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Nasal and Bronchial Testing as well as Treatment of Patients with Airway hyperresponsiveness and Inflammation focusing on the United airways concept

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Stockholm 2008

Published by Karolinska Institutet. Printed by [name of printer]

ISBN 978-91-7409-146-5

ABSTRACT

Allergic and non-allergic rhinitis and asthma is a global health problem on the increase that causes major illness and disability worldwide, and also results in a large financial burden on society. This thesis contains six papers, based on four different clinical studies on humans with allergic as well as non-allergic airways inflammation, such as neutrophil inflammation caused by exposure to swine dust, as well as chronic rhinosinusitis and concomitant asthma with and without NSAID intolerance. The purpose was to study different aspects of nasal and bronchial airways inflammation, with special focus on possible links between the upper and lower airways disease.

In paper I and II, the nasal and bronchial airway reactivity (sensitivity to histamine) in pollen allergy sufferers was detected after pollen exposure as well as out of pollen season. We found that the histamine-induced swelling of the nasal mucosa was increased in comparison to a control group of healthy individuals, without seasonal differences and without correlation to the bronchial histamine sensitivity. Counting the number of histamine-induced sneezes throughout the challenge test better than mucosal swelling correlated to the current allergic symptoms. The increase in histamine-induced nasal mucosal swelling out of pollen season was interpreted as a sign of "minimal persistent inflammation", a phenomenon previously described.

In paper III and IV healthy subjects were exposed to swine dust, with the purpose to study upper and lower airway inflammation. Swine dust exposure is a model previously developed for inducing airways inflammation in healthy subjects and until now used to mainly study the effects on the lower airways. We found the model useful for studying both nasal and bronchial inflammatory parameters, as swine dust exposure caused an increase in upper as well as lower airway sensitivity in healthy subjects, however without any mutual correlation. In this group of healthy volunteers, under inflammatory conditions after exposure to swine dust, we found an increase in histamine-induced nasal swelling and a decrease in bronchial function as measured by histamine-PC20. That is in contrast to the pollen allergy sufferers, where exposure to pollen did not induce such changes. Consequently, the airways reactivity was similar under inflammatory conditions in the allergy sufferers and in the group of healthy volunteers, and the main difference was under non-inflammatory conditions. Moreover, nasal lavage before a histamine challenge affected the outcome of the nasal mucosal swelling, microcirculation as well as nasal patency, which implies that this has to be

kept in mind when studies are designed, but it also sheds light on nasal irrigation as a treatment method of rhinitis. As we focused on measurements of the vascular response of the nasal mucosa, rhinostereometry was used in paper I-IV, and in the second study the method was developed to also contain a laser Doppler flowmeter, in order to perform simultaneous measurements of nasal mucosal swelling and microcirculation at the same area.

In paper V, this equipment was also evaluated as a method of detecting aspirin sensitivity throughout the nasal airways challenge test, but also to measure possible concomitant reactions of the nasal mucosa throughout the bronchial challenge. We found that the microcirculatory measurements differed between AIA and ATA patient groups, and that the main reaction, including vascular parameters as well as PNIF and symptom scores, occurred two hours or later after challenge. We therefore conclude that a three hour observation time after a nasal lysine-aspirin challenge may be recommended in order to improve the sensitivity of the method. We also found signs of a bronchio-nasal reflex in the AIA patients throughout the bronchial challenge test; with changes in the nasal microcirculation at the time they developed asthma.

In paper VI we evaluated the benefits of local steroid treatment and functional sinus surgery (FESS) on the upper and lower airways, in asthmatic subjects with nasal polyposis. Statistically significant improvements in mean asthma symptom scores and daily PEFR were noted despite the fact that the asthma in general was well controlled with inhaled corticosteroids. Moreover, in addition to a clear-cut improvement in the other nasal parameters, both subjective aspects of the olfactory function and the butanol test improved significantly. Together, these results further highlight FESS as a potent anti-inflammatory treatment method of the upper and lower airways, which could be considered early in the natural course of the disease with concomitant asthma, and a second-line treatment in nasal polyposis patients with a reduced sense of smell.

Finally, the findings that FESS has benefits on subjective and clinical asthma parameters, the detection of simultaneous changes in the nasal mucosal microcirculation at the time the AIA patients developed asthma throughout a bronchial lysine-ASA challenge, and the positive correlation between the number of sneezes following a histamine challenge and histamine-PC_{20}-PEF are findings that support the idea that the nose and bronchi are linked in a common functional airway system, also defined as “the united airways”.

LIST OF PUBLICATIONS

The present thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Karl-Gustav Kölbeck, Anders Ehnhage, Jan-Erik Juto
Nasal and Bronchial Histamine Reactivity in Patients with Allergic Rhinitis Out of Season. *Ann Allergy Asthma Immunol* 1999; 82:55–60.

II. Anders Ehnhage, Karl-Gustav Kölbeck, Björn Mossberg, Jan-Erik Juto
Nasal and Bronchial Histamine Responsiveness in Pollen-Exposed Patients with Seasonal Rhinitis. *ORL* 2002; 64:191–199.

III. Karl-Gustav Kölbeck, Anders Ehnhage, Jan-Erik Juto, Sune Forsberg, Hans Gyllenhammar, Lena Palmberg, Kjell Larsson.
Airway reactivity and exhaled NO following swine dust exposure in healthy volunteers. *Respiratory Medicine* 2000; 94: 1065–1072.

IV. Anders Ehnhage, Karl-Gustav Kölbeck, Pär Stjärne, Hans Grudemo, Jan-Erik Juto.
Swine dust exposure is a model for rapid induction of non-allergic neutrophil inflammation in the nasal mucosa of healthy volunteers, and the symptoms as well as the microcirculation are modified by nasal lavage. *Rhinology* 2007; 45(4): 292-298.

V. Anders Ehnhage A, Karl-Gustav Kölbeck, Jan-Erik Juto, Barbro Dahlén, Pär Stjärne.
Evaluation of nasal mucosal swelling and microcirculation throughout nasal and bronchial provocation tests with lysine-aspirin in asthmatics with nasal polyposis. *Clinical & Experimental Allergy*, Submitted.

VI. Anders Ehnhage, Petter Olsson, Karl-Gustav Kölbeck, Maria Skedinger, Barbro Dahlén, Martin Ålenius, Pär Stjärne.
Functional Endoscopic Sinus Surgery (FESS) improved asthma symptoms as well as PEFR and olfaction in patients with Nasal Polyposis. *Allergy*, in press.

LIST OF ABBREVIATIONS

ACE-inhibitors: Angiotensine-converting enzyme-inhibitors, a group of drugs with potent vasoconstrictor effects.

AE: Adverse event

AIA: Aspirin-intolerant asthmatics

ASA: Acetylsalicylic acid

ATA: Aspirin-tolerant asthmatics

BAL: Bronchial lavage

BH: Bronchial histamine provocation test

BHR Bronchial hyperresponsiveness

CMBC: The concentration of moving blood cells (in the microvascular blood flow)

COX: Cyclooxygenase enzyme

CRS: Chronic rhinosinusitis

CSF: Cerebrospinal fluid

CT: Computer tomography

ELISA: Enzyme-linked immunosorbent assay

EP^{3}OS: European position paper on rhinosinusitis and nasal polyps

FEV1: Forced expiratory volume for 1 second

FESS: Functional endoscopic sinus surgery

FPND: Fluticasone proprionate nasal drops

FVC: Forced vital capacity

GINA: The Global Initiative for Asthma

Histamine-PC20: The concentration of histamine producing a 20% decline in FEV_1

Histamine-PD-20: The dose of histamine producing a 20% decline in FEV_1

IAR: Intermittent allergic rhinitis

IgE: Immunoglobin E

ITT: Intent to treat

LPS: Lipopolysacharides

LT: Leukotriene

Lysine-ASA: Water soluble Acetylsalicylic acid

NAL: Nasal lavage

NAR: Nasal airway resistance

NARES: Persistent non-allergic rhinitis with eosinophilia syndrome

NO: Nitric Oxide

NSAID: Non-steroid anti-inflammatory drugs

OCS: Oral corticosteroids

PEF: Peak expiratory flow, measured by a spirometer

PEFR: Peak expiratory flow rate, measured by a portable apparatus.

PER: Persistent allergic rhinitis

PG: Prostaglandine

PNIF: Peak nasal inspiratory flow

PNEF: Peak nasal expiratory flow

PP: Per protocol

PU: Perfusion units

QoL: Quality of life

RAST: Radioallergosorbent test, a blood test for assessing the presence of specific IgE antibodies, used to determine what a person is allergic to.

RSM-LDF: A rhinostereometer with a connected laser Doppler flowmeter apparatus

SF-36: Study Short Form 36, a standardised questionnaire of the patient´s overall health status

SIT: Specific immunotherapy

VAS: Visual analogue scales

VC: Vital capacity

CONTENTS

1 INTRODUCTION

1.1 AIRWAY HYPERRESPONSIVENESS

The term *hyperresponsiveness* refers to exaggerated protective responses, which may arise in allergic and non-allergic rhinitis and asthma because of alterations in the normal response to non-specific triggers such as tobacco smoke, perfumes, temperature changes, strong odors, change of posture and hot drinks [1, 2]

In allergic rhinitis and asthma, hyperresponsiveness may also be involved in the phenomenon of *priming* to an allergen [3]. It is still unclear what causes hyperresponsiveness, whether it is in a specific cell or in a reflex arch with hypersensitive nerve endings [4].

Some believe that the central mechanisms like the facilitation of nerve transmissions are important [5], while others are more focused on environmental effects like stress on the brain [6]. Priming can be viewed as an augmentation of the acute allergic reaction to an allergen by repeated exposure. Although priming probably involves several mechanisms, it may be partly explained by an allergen-induced increased responsiveness to the products of an allergic reaction, such as histamine, leukotrienes, or prostaglandin D2 [1, 2].

Nasal hyperresponsiveness

This is an important feature of allergic and non-allergic rhinitis, and may be a result of changes in inflammatory cell behavior, sensory neural function, central information processing and gating, efferent messaging, end-organ sensitivity, or end-organ responsiveness in allergic and non-allergic airway diseases [2]. The typical symptoms are sneezing, nasal congestion and secretion, and in the allergic group more than 50% of patients with allergic rhinitis complain of nasal symptoms induced by irritants, with the prevalence of these complaints higher in perennial than seasonal disease [1]).

Different triggers, used in challenge tests for the detection of nasal hyperreactivity, are histamine, cold air, methacholine, ultrasonic nebulized distilled water, phentolamine, hyperosmolar fluids and capsaicin [1]. Histamine is perhaps the most commonly used trigger in nasal challenge tests, while methacoline is recommended in bronchial challenge tests (see below). Different stimuli will test different components.

Measurements of nasal responsiveness are at present mostly confined to research

studies investigating disease mechanisms in allergic and non-allergic rhinitis. The techniques are insufficiently standardized to be applied to multi-center clinical trials but could be used in limited-center studies to gain insight into the regulatory effects of different therapeutic modalities [2].

Bronchial hyperresponsiveness

For patients with symptoms consistent with asthma, but normal lung function, measurements of airway responsiveness are useful. These measurements reflect the "sensitivity" of the airways to factors that can cause asthma symptoms, sometimes called "triggers," and the test results are usually expressed as the provocative concentration (or dose) of the agonist causing a given fall (often 20%) in FEV1. The tests are sensitive for a diagnosis of asthma, but have limited specificity [7]. This is because bronchial hyperresponsiveness has been described in patients with allergic rhinitis [8], in healthy volunteers [9], and in those with airflow limitation caused by conditions other than asthma.

Mechanisms

There are several mechanisms behind bronchial hyperresponsiveness, such as an excessive contraction of lower airway smooth muscle cells [10] and later a thickening of the airway wall [11], sensory nerves may be sensitized by inflammation, which may lead to exaggerated bronchoconstriction in response to stimuli, and an uncoupling of airway contraction as a result of inflammatory changes in the airways [12]. Increased mast cell numbers in airway smooth muscle have been linked to airway hyperresponsiveness [12], and bronchial hyperresponsiveness may also result in a loss of the maximum plateau of contraction of the wall [13]. These are all factors that seem to contribute to bronchial hyperresponsiveness.

1.2 RHINITIS

Rhinitis is defined as an inflammation of the lining of the nose and is characterized by nasal symptoms including anterior or posterior rhinorrhoea, sneezing, nasal blockage and/or itching of the nose, and these symptoms occur during two or more consecutive days for more than 1 hour on most days [14].

Allergic rhinitis

Allergic rhinitis is the most common form of non-infectious rhinitis, and it is regarded as a major chronic respiratory disease due to its prevalence, impact on the quality of life, productivity and its association with asthma [14]. It is induced after allergen exposure and associated with an immunoglobulin E (IgE)- mediated immune response against allergens, often associated with ocular symptoms, and the link to asthma is strong [15], [16], [17], [18]. The symptoms include rhinorrhoea, nasal obstruction, nasal itching and sneezing, and allergic rhinitis is often associated with co-morbidities such as conjunctivitis, asthma and eczema.

Allergic rhinitis is subdivided into *Intermittent (IAR)* disease if symptoms are present less than four days a week and for less than four consecutive weeks, or *Persistent (PER)* disease if the symptoms are present more than four days a week and for more than four consecutive weeks. Allergic rhinitis is also classified based on the degree of severity.

Mild means that either sleep disturbance or impairment of daily activities, leisure and/or sport, school, or work are present, and that the present symptoms are not troublesome. *Moderate/severe* means that one or more of the symptoms above are present, and that the symptoms are troublesome [14].

Classically, outdoor allergens appear to constitute a greater risk for seasonal rhinitis than indoor allergens [19], and indoor allergens constitute a greater risk for asthma and perennial rhinitis [20]. The diagnosis is based on the history, heredity, allergic symptoms and other symptoms of atopy, diagnostic tests as immediate hypersensitivity skin tests or/and measurements of allergen-specific IgE in the blood [14].

Non-allergic rhinitis

Infectious

For infectious rhinitis, the term rhinosinusitis is usually used. Because the nasal and sinus mucosa form a continuum, the mucous membranes in the sinuses are most often involved in the primarily infected nasal mucosa. Common cold/acute *Viral* rhinosinusitis is defined as lasting <10 days. Acute *Bacterial* rhinosinusitis is defined by an increase in symptoms after 5 days or persistent rhinitis after 10 days with <12 weeks duration [14], [21].

Occupational

Occupational rhinitis is due to an airborne agent present in the workplace and may be allergic or a response to an irritant [22]. Examples are wood dust [23], furred animals (laboratory animals) [24], in farmers, swine house confinement buildings [25], etc., latex [26], grains (baker's rhinitis [27]).

Drug induced

The entire group of (non-Cox2 specific) *NSAIDs* often cross-react with *Aspirin* and induce rhinitis and asthma [28]. Other commonly used drugs known to induce rhinitis are: ACE-inhibitors and other vasoactive drugs, oral contraceptives [14], local α-adrenoreceptor antagonists (rhinitis medicamentosa)[29], preservatives in aqueous nasal, otic or ophthalmic products such as benzalkonium chloride [30, 31], intraocular or oral ophthalmic preparations of β-blockers and chlorpromazine [14]. Cocaine, which can be associated with rhinorrhoea, hyposmia and septal perforation [14].

Hormonal

Pregnancy rhinitis is so far the only clearly defined "hormonal rhinitis." [14]. However, the cause of pregnancy rhinitis is not simply elevated levels of estrogen or progesterone, but seems multifactorial [32]. Rhinitis of the menstrual cycle has been more described, although a solid picture is still lacking [33].

NARES (Persistent non-allergic rhinitis with eosinophilia syndrome)

This probably does not represent a disease entity on its own, thus it may be regarded as a subgroup of idiopathic rhinitis not associated with asthma, characterized by the presence of nasal eosinophilia and persistent symptoms of sneezing, itching, rhinorrhoea and sometimes also hyposmia, in the absence of demonstrable allergy. About half of the patients show bronchial non-specific hyperresponsiveness, and it has been suggested that in some patients it may represent an early stage of aspirin sensitivity [34, 35].

(Primary) Atrophic rhinitis

It is caused by a progressive atrophy of the mucosa as well as the underlying bone [36], which makes the cavity wide and open but full of crusts.

Emotional rhinitis:

Stress and sexual arousal have, probably autonomic, effects on the nose [37, 38].

"Senile rhinitis"

Symptoms from the nose are common in the elderly, however poorly described. The symptoms may range from simple watery rhinorrhoea without any impact on patency to frank obstruction [39].

Rhinitis caused by food and beverages

Alcoholic beverages

Alcohol commonly induces symptoms of rhinitis by unknown mechanisms, and in asthma only acute evoked by drinking alcohole, and sulphate additives in red wine have been proposed as a major mechanism. [40].

Food-induced rhinitis

Reactions to foodstuffs is most common as one of many symptoms of anaphylaxis [41], but hot red pepper and other spicy food can induce rhinorrhoea, probably because it contains capsaicin [42].

Rhinitis triggered by common irritants:

Tobacco smoke can cause an allergic-like inflammation in the nasal mucosa of non-atopic children [43].

Perfumes are other common irritants for the hyperreactive nasal mucosa, as well as *Cold dry air,* which is also used in provocation tests [44], and is also a problem for athletics in winter-sports [45].

Nasal measurement methods

Rhinomanometry

This is a well-established method for objective measurements of nasal airway resistance (NAR) during normal breathing, and is generally accepted as the standard technique of measuring NAR and assessing the patency of the nose [2, 21]. The measurements of NAR are performed by using either anterior or posterior rhinomanometry, and require patient cooperation [21].

Acoustic rhinometry

This method is based on a sonic echo technique, and measures internal nasal luminal volume, and the minimum cross-sectional area, and this is based on reflected sound waves. It requires a complex mathematical transformation, including several theoretical assumptions [46]. The outcome correlates well with that of CT [2],

Fig 1. Peak nasal inspiratory flow (PNIF)

PNIF (Peak nasal inspiratory flow) (Fig. 1)

PNIF is the technique best validated for home monitoring in clinical trials [2]. PNIF can be measured by using an anesthesia mask connected to a mini-Wright flow meter or a Youlten peak nasal inspiratory flow meter, and during the procedure, the patient places the mask over the nose and mouth and inspires forcefully through the nose with the lips closed. The equipment is portable, relatively inexpensive, and simple to use [2]. However, one disadvantage is that the nasal inspiratory peak flow is influenced by the lower airway as well as upper airway function, and there is a slight risk of nasal vestibular collapse, and it was found that 2 % of a group of allergy patients could not obtain PNIF measurements because of a total occlusion of the nose [47]. In one study PNIF and Peak nasal expiratory flow (PNEF) were more sensitive to mucosal changes throughout the histamine challenge than rhinomanometry, acoustic rhinometry and symptom scores [48].

PNEF (Peak nasal expiratory flow)

PNEF as well as PNIF are reported to offer highly reproducible values, but are effort-dependent, which might alter the variability [2]. Therefore, exacerbation in co-existing asthma may be a confounding factor when evaluating the nasal peak flow.

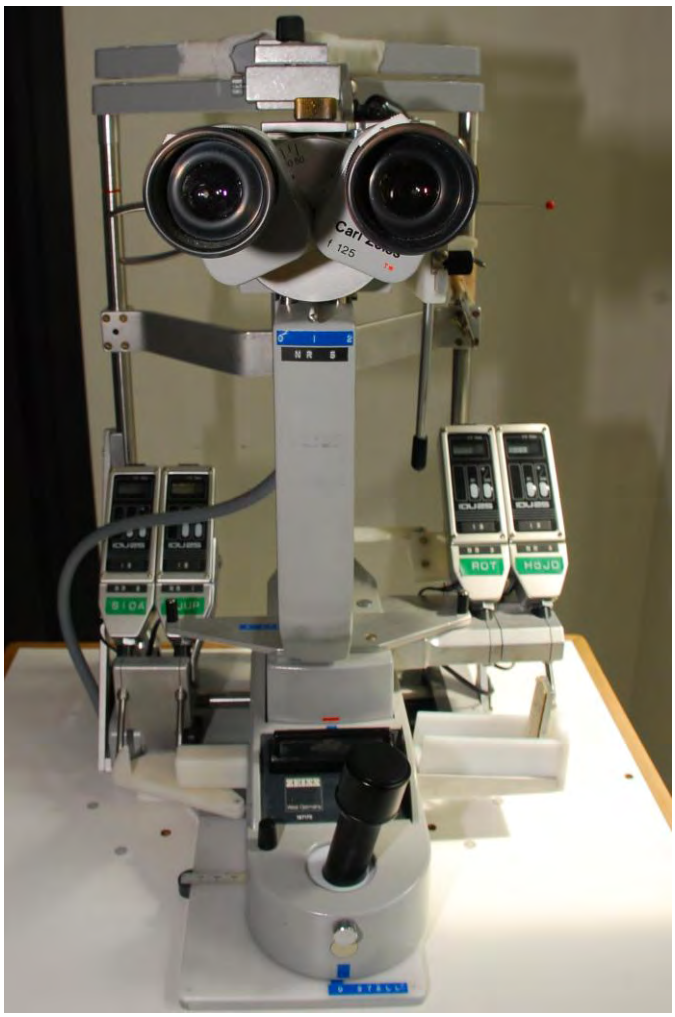


Fig. 2. The Rhinostereometer

Rhinostereometry and laser Doppler flowmetry (Fig. 2)

The rhinostereometer is used for direct measurements of nasal mucosal swelling of a certain area at the inferior turbinate. The recording device of the rhinostereometer consists of a surgical microscope placed on a micrometer table [49]. A laser Doppler flowmeter (Fig. 13) can record the microcirculatory blood flow in the the nasal mucosa, and when attached to a rhinostereometer this equipment (a RSM-LDF apparatus) can detect changes simultaneously in the deep (swelling) as well as in the superficial (microcirculation) part at a certain area of the nasal mucosa [50, 51].

Nasal lavage (NAL)

Nasal irrigation with isotonic saline is a useful method for collecting inflammatory cells and mediators of rhinitis, for different analyses. The technique is much simpler and easier than bronchial lavage [52].

Nasomucociliary clearance

The use of saccharin, dye or radioactive particles to measure mucociliary transit time, if altered, allows one to recognize early alterations of rhinosinusal homeostasis, with the advantage of considering the entire mucociliary system. Normally, the clearance time is <35 minutes, however if it is prolonged, it does not distinguish between primary or secondary causes of ciliary dysfunction. [2].

Olfaction threshold Tests

The estimation of olfactory thresholds by the evaluation of olfactory dysfunction in a number of serial dilutions of pure odorants, such as Butanol, have been used in a number of studies [53, 54]. Also scratch and sniff test, using patches impregnated with microencapsulated odorants, are available [55], such as the Zurich Smell Diskette test [56], the Barcelona Smell Test [57]. A combined supra-threshold detection and identification test has been devised as a cross-cultural tool in the European population [58].

VAS

Visual analogue scales are quantitative measures largely validated in rhinitis [59], in fact a single VAS scale was found to correlate very well with the severity assessed by ARIA [60].

Quality of life (QoL)

More and more attention has been paid to evaluating not only symptoms but also to the patient's health-related quality of life [14]. The QoL questionnaires can provide either general (generic) or disease-specific health assessment. However, QoL scales do not always correlate with the severity of nasal symptoms or findings [61].

The Medical Outcomes Study Short Form 36 [62] is an instrument for assessment of the patient´s overall health status. It contains standarized questions that are then

reduced to eight health status scores. Thus it offers the opportunity to compare the association of deficits in different diseases. It is by far the most widely used and well validated, and has also been used both pre- and post-operatively in chronic rhinosinusitis and in asthma patients [63] .

Treatment of rhinitis

The treatment of rhinitis includes allergen avoidance if possible, pharmacotherapy-including intranasal treatment with corticosteroids, chromones, H1- antihistamines, oral H1- antihistamines and anti-leukotrienes [14] . In Sweden the combination of oral H1- antihistamines and/or intranasal corticosteroids treatment is a common alternative. Specific immunotherapy (SIT) is mainly used as therapy for patients insufficiently controlled by conventional pharmacotherapy, but data is also presented that describes that SIT might reduce the risk for asthma development in children with bronchial asthma [64].

1.3 CHRONIC RHINOSINUSITIS

Rhinitis and sinusitis generally coexist and sinusitis always appears in the presence of concomitant rhinitis, and therefore the term rhinosinusitis is recommended. *The definition* of chronic rhinosinusitis is: "inflammation of the nose and the paranasal sinuses characterized by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior drip) +/- facial pain/pressure, +/- reduction or loss of smell and for >12 weeks". Chronic rhinosinusitis is divided into two main categories: with or without nasal polyps, and the former is the major part [21].

In **chronic rhinosinusitis without nasal polyps**, as in acute rhinosinusitis the predominant cells are neutrophils, although often with a small number of eosinophils, mast cells and basophils [21].

Nasal polyps, when found on examining the nose, are a sign of inflammation in the upper airways and can be associated with chronic bacterial infection, fungal infection, as well as with cystic fibrosis, ciliary dysfunction [65], Peutz-Jeghers syndrome [66], and *nasal polyposis* [21]. In this thesis, we make a distinction between nasal polyps, a symptom of nasal disease, and *nasal polyposis*, with bilateral diffuse nasal polyps, associated with asthma and NSAID intolerance [67].

Chronic rhinosinusitis with nasal polyposis

This is a chronic eosinophilic inflammatory disease in the nasal and paranasal mucosa, considered a subgroup of chronic rhinosinusitis at about 20% [68], and a disease in itself, with an unknown etiology [21].

The prevalence in the general population in Sweden has been found to be approximately 3% [69]..When no earlier sinus surgery has been performed, the definition is "polyps bilateral, endoscopically visualized in middle meatus", and when sinus surgery has been performed it is "bilateral pedunculated lesions as opposed to cobblestoned mucosa > 6 months after surgery on endoscopic examination" [21]. Nasal polyps and asthma, sometimes with aspirin hypersensitivity, are seen primarily in patients over 30 years old, and is rare in children [70]. Therefore children with nasal polyps should be assessed for cystic fibrosis and immotile cilia syndrome. [71].

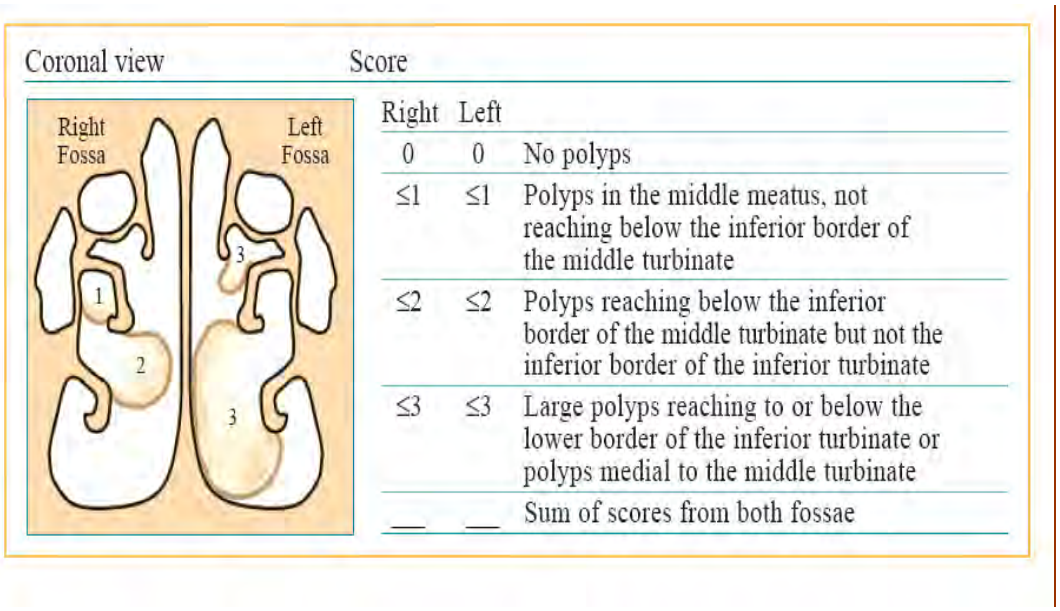


Fig. 3. Staging of nasal polyps

Staging of nasal polyposis (Fig. 3)

Staging is based upon endoscopic findings, after pretreatment with decongestants [72]. We used the system described in Fig. 3 (Paper VI) [53]. Different staging systems based on CT scanning have also been described, and the Lund Mackay system is commonly used. It has a score of 0-2 dependent upon the partial or complete absence of opacification of each sinus system and of the ostiomeatal complex, and the maximum score per side is 12 [73].

The subjective severity classification

A recent study has considered the relationship between subjective assessment instruments in chronic rhinosinusitis and has shown that 'mild' equates to a visual analogue score of 3 or less, 'moderate' to >3-7 and 'severe' to >7-10 [74].

Histopathology

The polyp tissue is characterized by edema, with supporting fibroblasts
and infiltrating inflammatory cells, predominantly activated eosinophils, indicating a major differences in the pathophysiology compared to chronic rhinosinusitis without nasal polyps with its neutrophil predominance [21].

Treatment of nasal polyposis:

Topical steroids spray have an effect on polyp size in nasal polyposis [75], congestion and quality of life [76]. Also objective methods as PNIF [75, 77], acoustic rhinometry [78], and rhinomanometry [79] have showed improvement after topical steroid treatment. It has also been showed that topical steroid treatment has effects on the eosinophil function [80], and the number of chemotactic cytokines in the nasal mucosa and polyp epithelial cells [81]..Also a short treatment period of 3 weeks has shown differences between treatment and placebo groups concerning symptoms but not polyp size [82].

Nasal drops are considered to be more effective than nasal spray and have a significant positive effect of the sense of smell [21]. However, the evidence is limited although some interesting papers on delivery systems have been published [83, 84].

Systemic steroid treatment has shown effects on polyp size, improvements of several symptoms, on anterior rhinomanometry, as well as on CT changes [85].

The safety of nasal and oral corticosteroids has been the subject of concern in medical literature since many patients with chronic sinus disease are prescribed these drugs. However, a clear distinction has to be made between the side effects of local nasal and oral corticosteroids treatment [21, 86].

Sinus surgery is recommended in selected patients who have not responded sufficiently well to medical therapy. It is difficult to generalise and standardize, as randomization and the type of treatment, including blinding, may cause ethical and technical problems for evidence-based evaluation. Moreover, the nasal polyp patient group is not homogenous, with subgroups, such as concomitant NSAID intolerance, bronchial

asthma, allergy as well as other concurrent systemic diseases. The number and types of previous surgeries might vary as well as the pre- and post-surgery medical treatment, and age as well as duration of the disease. Finally, there are several technical alternatives, including simple nasal polypectomy, using a snare (a collective term for surgical techniques already used before the development of functional sinus surgery), or functional surgical procedures, and the surgery might also be characterized as limited, extended or radical [21].

The use of functional endoscopic sinus surgery has more and more become dominant as the most common technique, and over the last few years the use of microdebriders has been common. It is an instrument originally developed for arthroscopic surgery, with a suction-based powered instrument containing a blunt end and a oscillating or rotating blade, thereby cutting and removing only tissue suctioned into the instrument opening while blood and tissue debris are removed [21]. When Dalziel and co-workers performed a meta-analysis, 477 articles were scanned or evaluated about the clinical effectiveness of FESS on nasal polyposis patients, the level of evidence was low in general [87]. The authors concluded that FESS may offer some advantages in safety and effectiveness over comparative techniques, but the wide variation in reported results and methodological shortcomings of studies limit the certainty of these conclusions. The wide variation in complication rates suggests the need for an audit of existing practice. Additional high-quality studies with a fuller description of potential confounding factors and effect modifiers will help to define the effectiveness of FESS more clearly. However, our clinical impression is that with modern treatments, the patients who require surgery many times every year have disappeared.

A preoperative CT-scan is nowadays standard in the preoperative assessment, as a tool for avoiding the loss of landmarks which can generate known complications in surgery such as CSF leak, meningitis, intracranial lesions, diplopia, intraorbital hematoma, loss of vision and even death. According to EP^{3}OS 2007, extended surgery has not proved better results than limited surgical procedures, and it appears reasonable to tailor the extent of surgery to the extent of the disease. Extensive polyps, NSAID-intolerance, asthma and cystic fibrosis are predictors for revision surgery, and today surgery is only indicated if medical treatment is not sufficiently effective [21]. The effect of surgical CRS treatment on concomitant asthma, on patients with NSAID- intolerance as well as on allergy has not been clearly shown, and the level of evidence is low [21].

1.4 ASTHMA

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, tightness of the chest, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment [88]. The predominant feature of the clinical history is episodic shortness of breath, particularly at night, often accompanied by coughing, wheezing, and chest tightness [89].

The main physiological feature of asthma is episodic airway obstruction characterized by expiratory airflow limitation (Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma (GINA), 2006. Available from www.ginasthma.org. Date last updated. 2006).

The inflammation of the bronchial mucosa in asthma is persistent even though symptoms are episodic, with a characteristic pattern similar to that in allergic rhinitis, although in contrast to in the nose, where histamine is a key mediator, have the Leukotrienes an important role, in particular in aspirin-intolerant asthmatics (AIA)[90].

As in rhinitis, asthma is characterized by airway hyperresponsiveness, often atopy and allergic sensitization [91]. However, the anatomic differences, i.e. smooth muscles, results in the characteristic intermittent narrowing of the bronchi in asthmatics in contrast to in patients with rhinitis. There is data to support the hypothesis that the variable component of airway hyperresponsiveness reflects airway inflammation and is associated with current exposure, asthma activity, and asthma severity, and some patients with mild and/or episodic asthma may only exhibit this component of airway hyperresponsiveness[4].

Asthma associated with rhinitis may occur intermittently, with the patient being entirely asymptomatic between seasons or it may involve seasonal aggravation of asthma symptoms or a background of persistent asthma [16, 92].

Diagnosis

The diagnosis of asthma is usually based on the presence of the characteristic symptoms and the demonstration of variable airway obstruction and/ or airway hyperrresponsiveness. Variable airway obstruction is documented either by *spirometry* (Fig. 4) with a reversability test, or daily peak flow (*PEFR*) measurements (Fig 5).

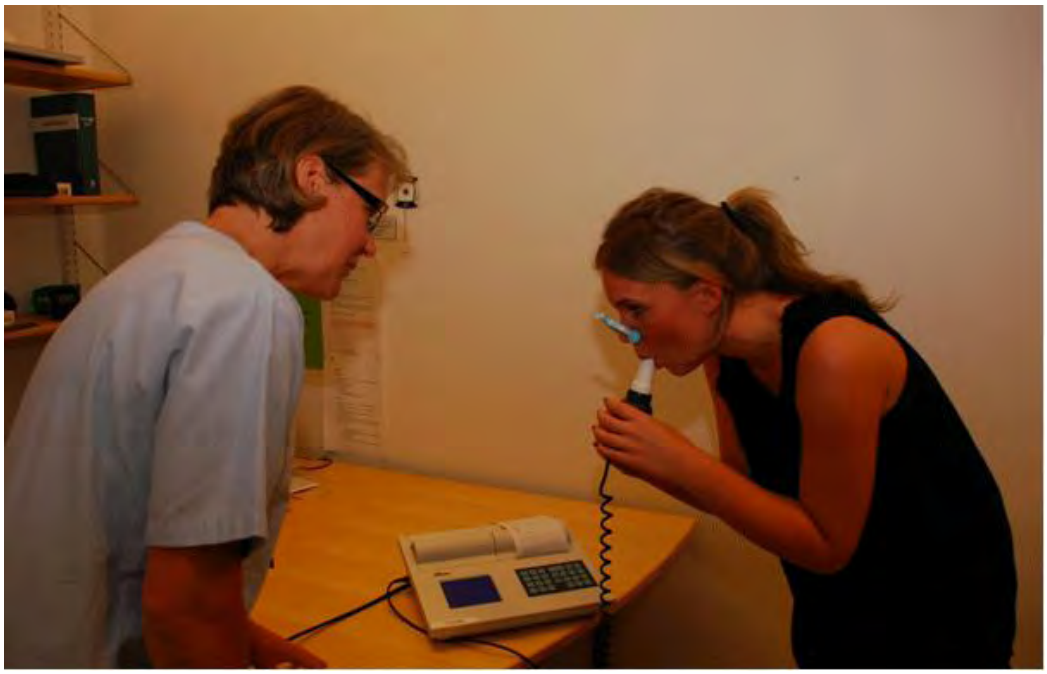


Fig. 4. Spirometry

Spirometry

In particularly the measurement of forced expiratory volume for 1 second (*FEV1*), but also forced vital capacity (*FVC*), and peak expiratory flow (*PEF*) measurement, are two methods that have gained widespread acceptance for use. The degree of reversibility in FEV1 indicates a diagnosis of asthma, and is generally accepted as ≥ 12% and ≥ 200 ml from the pre-bronchodilator value [93], Standardization of Spirometry 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995;**152**(3):1107-1136).

Airway hyperresponsiveness is assessed by inhalation of methacoline or histamine in challenge tests [1, 4].

Fig. 5. The PEFR apparatus

PEFR

This is a simple and cheap apparatus used by patients in their home, and is effort-dependent. It is used in the morning before treatment is takenand at night. One method of describing diurnal PEFR variability is as the amplitude (the difference between the maximum and the minimum value for the day), expressed as a percentage of the mean daily PEFR value, and averaged over 1-2 weeks [94]. Another method of describing PEFR variability is the minimum morning pre-bronchodilator PEFR over 1 week, expressed as a percent of the recent best (Min%Max) (Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma (GINA), 2006. Available from www.ginasthma.org. Date last updated. 2006).

Airway obstruction in asthma

The predominant mechanism behind this intermittent narrowing of bronchi is due to airway smooth muscle contraction in response to broncho-constrictor mediators and neurotransmitters. Mucus hypersecretion also contributes to this narrowing and results from increased numbers of goblet cells in the airway epithelium and the increased size of submucosal glands [95]. Airway edema is due to increased microvascular leakage, and this may be particularly important during acute exacerbations. Mucus secretion and inflammatory exudates may lead to “luminal plugging” [96]. Subepithelial fibrosis

results from the deposition of collagen fibers and proteoglycans under the basement membrane [97]. It is seen in all asthmatic patients, even before the onset of symptoms but it may be influenced by treatment. Blood vessels in airway walls increase the influence of growth factors and may contribute to increased airway wall thickness [13]. Permanent airway thickening is due to structural changes, often termed "remodeling", which may be reflected in some patients with severe asthma, developing a progressive airflow limitation that is not fully reversible with currently available therapy [98].

Measurements of bronchial hyperresponsiveness

Based on the mechanism of action, direct or indirect stimuli may contribute to establishing a diagnosis of asthma [99], and to detecting bronchial hyperresponsiveness. These stimuli are categorized as direct or indirect. Direct stimuli cause airway smooth muscle contraction by activating specific receptors on its cell membranes, and they are *methacholine* or *histamine*. Methacholine mimics the effect of endogenous acetylcholine on airway smooth muscle muscarinic receptors [4].

Histamine has almost an equivalent molecule, but has the disadvantage of side effects (headache, sore throat, itching) and less reproducibility (Standardization of Spirometry 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995;**152**(3):1107-1136). Therefore methacoline is more commonly used for bronchial challenges.

Measurements of Nitric Oxide (NO)

Nitric oxide is produced in the upper and lower respiratory tract, with the highest concentrations in the sinuses. The technique is used as an indicator of the current degree of inflammation [100], being high with inflammation and low in ciliary dyskinesia. It requires little patient co-operation and is quick.

For nasal measurements, the availability of measuring equipment at present limits its use, but it is getting more and more common in clinical practice for bronchial measurements in pulmonary departments.

VAS and quality of life questionnaires are used for evaluating patients suffering from asthma, rhinitis and chronic rhinosinusitis, and are further described in the Rhinitis part of the Introduction.

Asthma treatment

The last updated GINA version 2006, recommends a change in approach to asthma management, with asthma control, rather than asthma severity, being the focus of treatment decisions [88].

Glucocorticoids

Inhaled glucocorticosteroids are currently the most effective anti-inflammatory medications for the treatment of persistent asthma (Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma (GINA), 2006. Available from www.ginasthma.org. Date last updated. 2006). Studies have demonstrated their efficacy in reducing asthma symptoms, improving quality of life [101], and lung function, and reducing airway hyperresponsiveness [102]. Inhaled glucocorticosteroids are the most effective controller medications currently available (Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma (GINA), 2006. Available from www.ginasthma.org. Date last updated. 2006).

Oral glucocorticosteroid treatment in asthma therapy is commonly used to treat exacerbations (Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma (GINA), 2006. Available from www.ginasthma.org. Date last updated. 2006).

ß$_2$-agonists

Rapid-acting inhaled ß$_2$-agonists are the medications of choice for relief of broncho-constriction and for the pretreatment of exercise-induced broncho-constriction (Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma (GINA), 2006. Available from www.ginasthma.org. Date last updated. 2006).

Long-acting inhaled ß$_2$-agonists are most effective when combined with inhaled glucocorticosteroids [103]

Leukotriene modifiers

has a small and variable bronchodilator effect, reduce symptoms including cough [104], improve lung function, and reduce airway inflammation and asthma exacerbations [105].

1.5 AIRWAYS INFLAMMATORY CELLS AND MEDIATORS

Inflammatory cells

Mast cells

release bronchoconstrictor mediators (histamine, cysteinyl leukotrienes, prostaglandin D2 [106], by allergens through high-affinity IgE-receptors, and by osmotic stimuli as in exercise-induced bronchoconstriction. Increased numbers of mast cells in airway smooth muscle may be linked to airway hyperresponsiveness.

Eosinophils

present in increased numbers in the airways, release basic proteins that may damage airway epithelial cells. They may also have a role in the release of growth factors and airway remodeling [107].

T lymphocytes

are present in increased numbers in the airways, releasing specific cytokines that induce eosinophilic inflammation and IgE production by B- lymphocytes [108].

Macrophages

are found in increased numbers in the airways and may be activated by allergens through IgE receptors to release inflammatory mediators and cytokines [109].

Dendritic cells

sample allergens from the airway surface and migrate to regional lymph nodes, where they interact with regulatory T cells and ultimately stimulate the production of Th2 cells [110]. *Neutrophil* numbers increase in airways patients with severe asthma, with an uncertain role [111].

Key Mediators of Asthma

Cysteinyl leukotrienes are potent bronchoconstrictors and proinflammatory mediators mainly derived from mast cells and eosinophils. They are

the only mediator whose inhibition has been associated with an improvement in lung

function and asthma symptoms [113].

Prostaglandin D2

is a bronchoconstrictor derived predominantly from mast cells and is involved in Th2 cell recruitment to the airways [114].

Chemokines

are important in the recruitment of inflammatory cells into the airways and are mainly expressed in airway epithelial cells [115].

Cytokines

induce an inflammatory response in asthma and determine its severity (Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma (GINA), 2006. Available from www.ginasthma.org. Date last updated. 2006). IL-1 and TNF-oc amplify the inflammatory response, and GM-CSF prolongs eosinophil survival. IL-5 is required for eosinophil differentiation and survival [116], IL-4 is important for Th2 cell differentiation [116], and IL-13 is needed for IgE formation.

Nitric oxide (NO)

is a potent vasodilator, produced predominantly from the action of inducible nitric oxide synthase in airway epithelial cells [117], and exhaled NO is reportedly associated with the presence of inflammation in asthma [100].

Histamine

is released from mast cells and contributes to some extent to bronchoconstriction and to the inflammatory response[112].

1.6 THREE DIFFERENT TYPES OF INFLAMMATION STUDIED IN THIS THESIS

Fig. 6. Birch pollen

The allergic inflammation

Allergic rhinitis and asthma are induced after allergen exposure by an immunoglobin E (IgE)-mediated inflammation of the airways membranes [14]. When an allergen interacts with membrane-bound IgE on the surface of mast cells in a sensitized individual, this triggers degranulation and the allergic reaction take place [118]. Th2 lymphocytes regulate the reaction by the release of cytokines (IL-3, IL-4 and IL-5), which in turn effectuate the allergic reaction by their influence on mast cells, B-cells and eosinophils [107], and the concomitant following release of mediators, such as histamine [119, 120],

The allergic reaction after an allergen exposure contains an immediate reaction and a late phase reaction. The immediate reaction occurs within 15 minutes to about two hours, and is mediated by pro-inflammatory mediators, among which histamine, when released from mast cells and basophils, plays a central role in the nose. The release of histamine leads to both a sensory reflex, which produces pruritus and sneezing, and a nasonasal central reflex with a cholinerg efferent arm leading to activation of submucosal glands and the symptom of rhinorrhoea, as well as to some extent also to

vasodiation [112], while the typical immediate reaction of the lower airways is bronchospasm. In contrast to in the nose, Leukotrienes, are the predominant mediators in the bronchi [121]. Leukotrienes, Tryptase and prostaglandines also participate as mediators of the immediate reaction, and this leads in turn to increased nerve activation, plasma leakage, activation of adhesion molecules and migration of inflammatory cells [122].

The late-phase reaction occurs about four to ten hours after exposure (with a maximum about seven to eight hours after exposure), and is characterized by a cellular recruitment and activation by cytokines, with an inflammatory cell migration and an adhesion cascade, creating the symptoms of nasal congestion, nasal hyperreactivity and sometimes hyposmia from the nose, and the bronchial symptoms are a longer-lasting bronchospasm [10, 123], as well as increased secretion and edema of the bronchial mucosa. Interestingly, the nasal symptoms of the immediate and late-phase reaction correlate in time with the corresponding bronchial reaction as measured by a decrease in FEV1 (Book: Lemanske RF Jr, In: *Asthma and Rhinitis*. 2nd ed. Oxford: Blackwell Science; 2000:1172-1185). If the allergen exposure continues, this might lead to priming [3], with increased cell concentration, remodeling [11], and a minimal persistent inflammation [124]

Neutrophil inflammation after exposure to swine dust (Papers III and IV)

Fig. 7. A swine dust producer

In healthy previously unexposed subjects, a short time exposure to swine dust commonly causes an intense airway inflammation, often accompanied by symptoms of malaise, chills, fever and headache (organic dust toxic syndrome) [25, 125],. Analyses of NAL and BAL after three hours working in a swine confinement building have shown an influx of inflammatory cells into the airways with increases in the number of cells, i.e. 40 to 50-fold increases in neutrophils [125], a doubling of alveolar macrophage numbers and a three-fold increase in lymphocyte numbers [25, 125], and in the concentrations of plasma proteins, i.e., α-2 macroglobulin, albumin and transferrin [126], in acute-phase proteins and IL-6 [25], and several cytokines such as IL-8, IL-1α, IL-1β, IL-6, TNF-α [125, 127], (Paper III). It has also been observed that inhaled and intranasally-instilled fluticasone proprionate, attenuated the inflammatory response, attenuated the plasma protein leakage, the IL-8, IL-6, and TNFα levels, as well as an increase in body temperature seen after exposure, although the increased bronchial methacholine sensitivity was not altered by the use of this steroid [128]. Therefore, this model with weighing pigs in a large confinement building for three hours with exposure to a certain amount of dust, has been proved useful for studying the behavior of otherwise healthy airway mucosa under inflammatory conditions.

The mechanism behind the strong potential of the method of inducing a neutrophil airways inflammation has not yet been fully understood. The airborne swine dust contains particles from crushed feed, swine dander and micro-organisms from feces e.g. mainly gram-positive, such as Enterococci [129], and the cell walls of these bacteria contain elements that may influence airway cells, such as muramyl peptide, peptidoglycan, teichuronic acid and formyl-methio-1-lecyl-phenylalanine (fMLP)-like peptides [130].

However, swine dust also contains gram-negative bacteria and endotoxins [25, 125]. which can cause a strong neutrophil inflammation of the airways.

Lipopolysacharide (LPS), which is present only in gram-negative bacteria, correlated with symptoms and with an increase in BHR and a decrease in vital capacity (VC) after swine dust exposure [130]. It has therefore been proposed that the latter are responsible for this type of inflammation [25]. On the other hand, a correlation between the concentration of airborne bacterial peptidoglycan and the increase in neutrophils in BAL fluid has been found, indicating that the bacterial content of the airborne dust plays a central role in the development of the massive inflammation [127]. In vitro experiments have also shown that LPS are probably not the sole agents, multiple agents from both gram-positive and gram-negative bacteria play a role in the induction of airway inflammation and general health effects in persons exposed to swine dust, rather than a hypothesis that all such effects are due to LPS [130], and although LPS certainly plays a fundamental role, the data cannot be interpreted as solely a consequence of exposure to endotoxins [125].

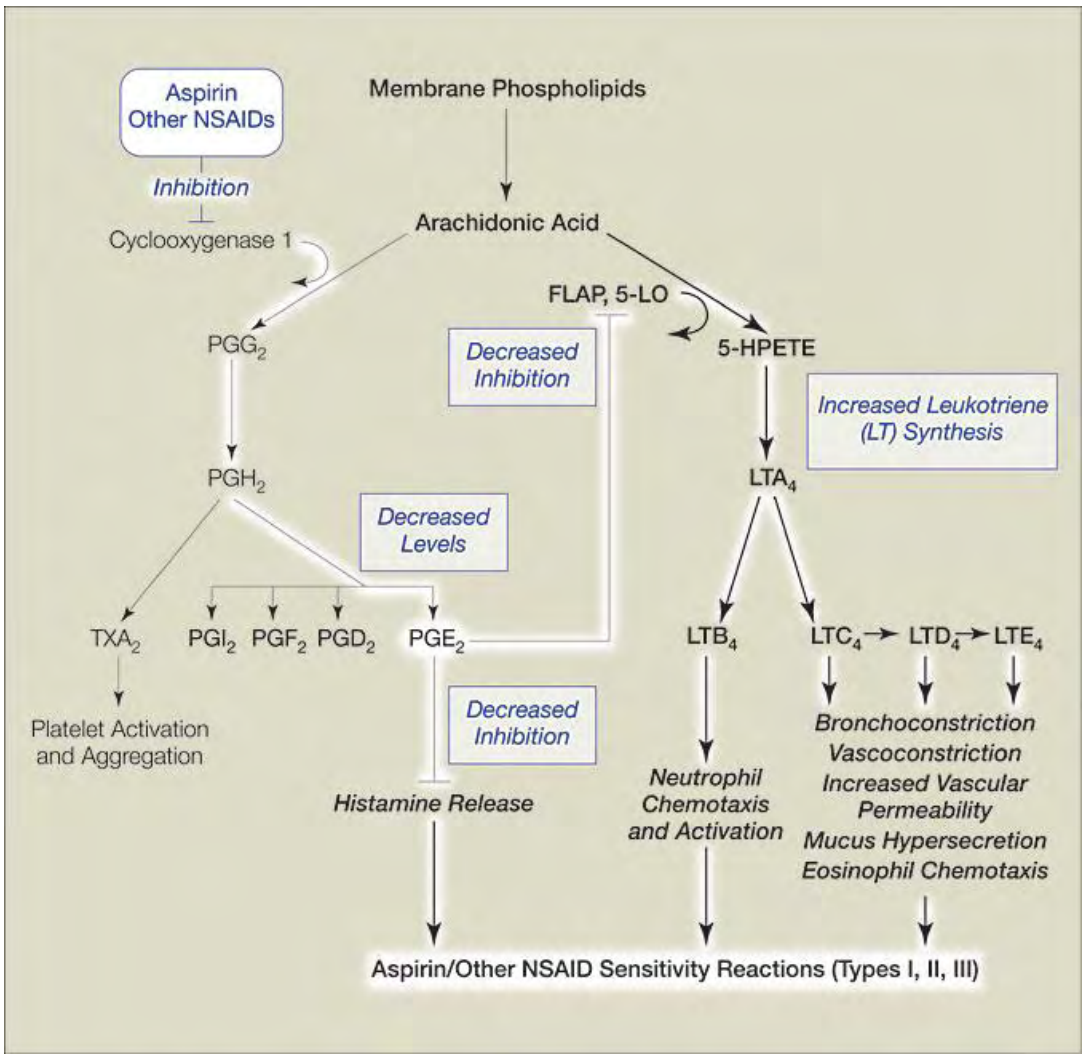


Fig. 8. Inhibition of cyclooxygenase-1 (COX1)
Raghava et al. *JAMA.* 2004;292:3017-3023.

Aspirin/NSAID-intolerance (Paper V)

The presence of aspirin-intolerance in a patient with nasal polyposis is associated with a high recurrence of nasal polyps, often with a co-existence of severe bronchial asthma (Samter´s triade) [131]. The precipitation of asthma attacks and rhinitis by aspirin and other NSAID is a prominent feature of the syndrome. Sometimes the reactions are generalized, and fatalities occur when patients are inadvertently prescribed anti-phlogistic remedies or obtain painkillers over the counter. The NSAID-intolerant group are characterized by an extensive polyposis often involving all the sinuses, and frequent need for endoscopic sinus surgery. The prevalence of nasal polyposis in aspirin-sensitive asthmatics may be as high as 60-70%, as compared to less than 10% in the population of aspirin-tolerant asthmatics [132].

The natural course of this disease is typically in the second or third decade of life with the development of rhinitis, subsequently followed by asthma and aggravated nasal symptoms including nasal congestion, recurrent nasal polyposis and loss of sense of smell. The first episode of an aspirin-/NSAID induced reaction is often unexpected. Occasionally it may present as a first attack of acute asthma (rhinitis) but more commonly the reaction occurs when the respiratory symptoms have been present for some years [70]).

The mechanism is still unclear. An oral intake of aspirin or other cross-reacting non-steroidal anti-inflammatory drugs (NSAIDs induces acute adverse reactions in ASA-sensitive patients. The phenomenon of cross-reactivity between structurally-unrelated chemical compounds makes an immunological mechanism unlikely. It is well documented that AIA have an increased urinary excretion of cysteinyl-leukotrienes ([133, 134], mediated by an inhibition of the cyclo-oxygenase enzyme (COX-1), which is the constitutive enzyme responsible for synthesizing prostaglandines. There is also an association with eosinophilia [67], where the eosinophil represents a potential producer of leukotrienes. Furthermore the mast cell has been defined as an important player in the intolerance reaction, and is the likely source of PGD2 and histamine[28]. The dramatic effect of COX-inhibitors in AIA patients is believed to be related to an abnormal dependency on the protective anti-inflammatory action of PGE2 [135, 136], resulting in mast cell activation upon removal of endogenous PGE2.

The concept of excessive leukotriene formation and its contribution to chronic airways obstruction in AIA is supported by successful treatment trials with anti-leukotriene drugs [137, 138]. However, in patients with nasal polyposis there are few studies that show that leukotriene-receptor-antagonists have significant effects on upper airways symptoms [139].

1.7 UNITED AIRWAYS

Several studies have pointed out the connection between inflammatory diseases in the upper and lower airways, which has lead to establishment of the concept, " the united airways" or "the unified airways", etc.

Allergic rhinitis and asthma are both disorders that share a common inflammatory process, share common inflammatory cells and mediators, that have been linked epidemiologically, pathophysiologically, and clinically as "one airway disease" [14].

The majority of patients with asthma have a history or evidence of rhinitis and up to 30% of patients with persistent rhinitis have or will develop asthma [140]. In patients with nasal polyposis, asthma was found in 30% in those referred to ENT departments, and in more than 70% of those referred to allergy departments [141], and 29%-70% of patients with nasal polyps may have asthma [141, 142]. Rhinitis and asthma also share several risk factors: common indoor and outdoor allergens such as house dust mites, animal dander, and less commonly, pollen affecting both the nose and bronchi [143], occupational sensitizers [144], and non-specific factors like aspirin.

Rhinitis frequently precedes asthma, and is both a risk factor for the development of asthma [145]*),* and is associated with increased severity and health resource use in patients with concomitant asthma [92]. Patients with allergic rhinitis have signs of inflammation in the bronchii with eosinophilia, shedding in the bronchial epithelium, thickening of the basal membrane in biopsies from bronchial tissue, but also eosinophilia in peripheral blood [14, 146], as well as an increased bronchial responsiveness [147-149]. An allergen challenge to the nose in AR patients increased the bronchial hyperresponsiveness [150].

Many patients with allergic asthma also have an inflammation of the nasal mucosa with correlation between the eosinophil concentration in the nasal and bronchial mucosa [151] and in both allergic asthmatics and rhinitis patients, sputum eosinophil and Eosinophilic Cation Protein (ECP) levels increased significantly after bronchial allergen provocation [152]. A bronchial allergen challenge in allergy patients has been shown to increase eosinophil levels in both bronchial and nasal tissues, as well as in peripheral blood, i.e., local as well as systemic effects [153]. When treating the rhinitis adequately in a group of asthmatics, the asthma exacerbations decreased significantly and the costs decreased by 50% [154].A concomitant diagnosis of allergic rhinitis was a significant predictor of higher annual costs for asthma medications in this patient group [92].

Both asthma and rhinitis are inflammatory disorders of the airway with very much in common, but there are some differences between the two conditions in mechanisms, clinical features, and treatment approach. Because there are differences in the structure of the mucosa in the upper and lower airways, with a greater vascularity of nasal tissue, the presence of smooth muscle in the bronchi, and a greater degree of epithelial shedding in the lungs, an allergic inflammation in these target tissues induces the expression of a different set of symptoms in allergic rhinitis and asthma, including

bronchoconstriction in the lungs and vascular engorgement leading to nasal obstruction in the nose [15]. Although the inflammation of the nasal and bronchial mucosa may be similar, nasal obstruction is largely due to hyperemia in rhinitis, while airway smooth muscle contraction plays a dominant role in asthma [155]. Often the medical treatment includes the same drugs for asthma and rhinitis. Glucocorticosteroids as well as leukotriene modifiers and anticholinergics can be effective in both conditions. However, H1-antagonists are selectively effective against rhinitis (e.g.,) as are β-2-agonists against asthma [156]. The use of intra-nasal glucocorticosteroids for concurrent rhinitis has been found to have limited benefits in improving asthma and reducing asthma morbidity in some but not all studies [157, 158]. Leukotriene modifiers [159], allergen-specific immunotherapy and anti-IgE therapy [14], are effective in both conditions, and the treatment of rhinitis may improve asthma symptoms [160, 161], and it has been showed that specific immunotherapy in children with allergic rhinitis has the potential to reduce the risk of developing bronchial asthma [162].

2 AIMS

1. To investigate airway histamine sensitivity of the nasal mucosa, as measured by nasal mucosal swelling with RSM and symptom scores, in pollen allergy sufferers out of season, during pollen season and after a single nasal pollen provocation out of season.
2. To investigate airway histamine sensitivity of the nasal mucosa as measured by RSM-LDF and symptom scores in healthy subjects before and after exposure to swine dust.
3. To evaluate possible effects of nasal saline lavage on nasal mucosal swelling and microcirculation, as well as on symptom scores throughout the histamine challenge test.
4. To evaluate RSM-LDF as a method for non-invasive simultaneous measurements of nasal mucosal swelling and the microcirculation at a certain area of the inferior turbinate.
5. To study the nasal and bronchial reaction time course for 180 minutes after a single nasal spray dose of lysine-aspirin in order to investigate a possible late reactivity in the nasal mucosa, and to evaluate a possible reaction in the nasal mucosa upon bronchial challenge.
6. To evaluate RSM-LDF as objective methods for the diagnosis of aspirin intolerance in nasal challenge tests, and compare these to the already established methods PNIF and symptom scores.
7. To investigate a possible correlation between bronchial and nasal histamine responses in pollen allergy sufferers in season, in healthy subjects with a continuous neutrophil inflammation after exposure to swine dust, and between bronchial and nasal responses to lysine-aspirin in AIA patients.
8. To study the effects of FESS and local treatment with Fluticasone proprionate on clinical and objective bronchial and nasal parameters in patients with nasal polyposis and asthma.

3 PATIENTS AND METHODS

3.1 PATIENTS

Papers 1 and II

Twelve otherwise healthy patients (five women), mean age 31.7 (range 19-43) years, were selected from the Department of Pulmonary and Allergic Diseases where they had been examined for seasonal rhino-conjunctival distress, but not found to have chronic bronchial asthma. They all completed a standardized questionnaire concerning allergic symptoms from the upper respiratory tract. They had a positive skin prick test for birch and/or timothy, but were negative for allergens that commonly cause perennial allergy in Sweden, such as cat, dog, horse dander, house dust mites and molds. Depending on the history and skin-prick tests, five patients were divided into a "birch" and seven into a "timothy" group. These allergies were further confirmed by RAST testing (Pharmacia, Sweden).

Papers III and VI

Seventeen healthy non-smokers participated in the study. They had had no previous exposure to farm dust, or history of chronic rhinitis, allergy, asthma or signs of airway disease on a physical examination of the nose and lungs.

The subjects were divided into two groups in order to evaluate the local nasal effects of a nasal histamine challenge test and nasal lavage respectively. Therefore, in Group 1 (4 men and 4 women, mean age 25, range 18-32 years) the nasal histamine challenge test was done first and was followed within 10 minutes by a nasal lavage. In Group 2 (4 men and 5 women, mean age 27, range 17-32 years), the order was reversed.

Paper V

Eighteen patients, with asthma and nasal polyposis, (seven women) aged 30-61 years, participated in the study. They were all selected from the Department of Pulmonary and Allergic Diseases or/and the ENT department. Eleven of the patients had a history suggesting NSAID-induced asthma, in two patients the history only slightly indicated this diagnosis, and five patients had previously had no adverse events due to NSAID.

Mean asthma duration (doctor´s diagnosis and in general also when starting asthma treatment) was 17.4 years in the AIA group and 22.0 years in the ATA (aspirin tolerant asthmatic) group (Table 1).

Paper VI

Eighty-two patients, aged 18 years or older (range 19-78 years), with a diagnosis of bilateral nasal polyposis and asthma, were recruited from the outpatient clinic at the ENT department of the Karolinska University Hospital, Huddinge, Stockholm, Sweden, and assessed for eligibility. The asthma diagnosis was based on history and lung function tests, as assessed by a pulmonologist, and all but one were on inhalation steroids at the start of the study. They were also required to have bilateral nasal polyps upon endoscopic examination by an ENT specialist. After wash-out for nasal steroids, 68 patients were randomized for further participation. All but one were on inhalation steroids at the start of the study.

3.2 METHODS

Study design

Papers I and II

In the first study, both nasal and bronchial histamine sensitivity were studied throughout and out of pollen season in 12 pollen allergy patients with the major symptom being rhinoconjunctivitis, and the nasal test was performed one day before the bronchial one. We also wanted to study whether repeated allergen exposure would increase nasal histamine sensitivity (the priming effect) as compared to a single pollen exposure. Therefore, 24 hours after a nasal allergen provocation, a separate single nasal histamine challenge test was performed out of pollen season. In total, the patients underwent three nasal and two bronchial histamine challenge tests in this study.

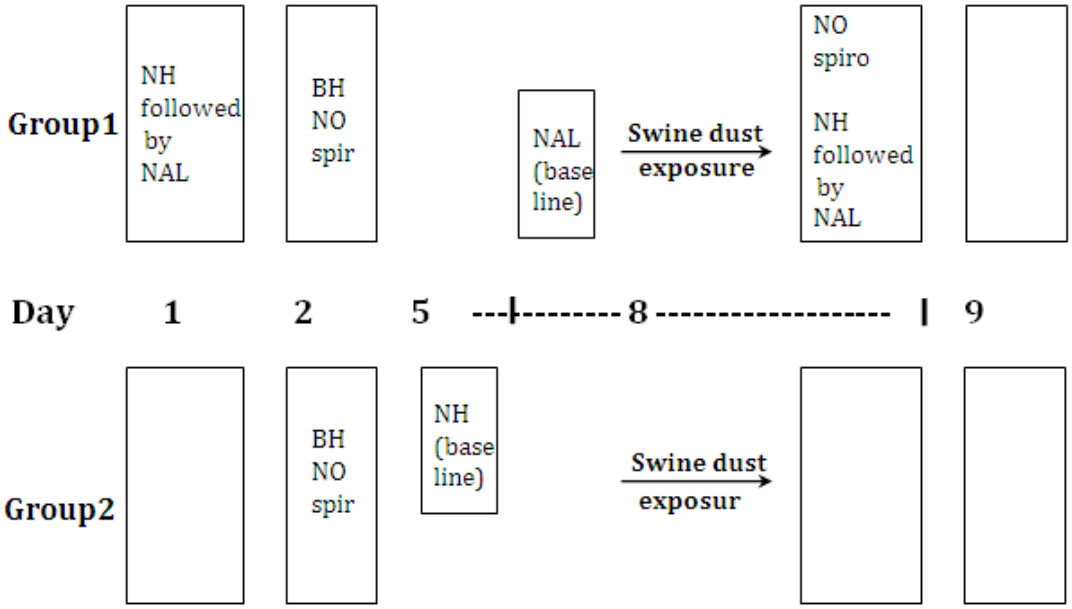


Fig 9. Study design Paper III.
NAL: Nasal lavage; NH: nasal histamine provocation test; NO: measurement of exhaled nitric oxide; spiro: dynamic spirometry; BH: bronchial histamine provocation test.

Papers III and IV (Fig. 9)

A group of 17 healthy volunteers without any known allergy or other symptoms from the airways, participated. To also study the influence of nasal lavage on nasal histamine challenge test and the reverse, the subjects were randomized into two groups. *Group 1* had a nasal histamine challenge test before nasal lavage to study the effects of inflammation on nasal mucosal swelling and on the microcirculation throughout the histamine challenge, and *Group 2* first had nasal lavage and immediately thereafter a nasal histamine challenge test, to study the influence of nasal lavage on the outcome of these parameters throughout the histamine challenge test. The nasal measurements throughout the nasal histamine challenge were performed using RSM-LDF for simultaneous measurements of nasal mucosal swelling and microcirculation, as well as calculating the number of sneezes, and the corresponding bronchial histamine challenge was evaluated by dynamic spirometry, a bronchial histamine challenge and measuring NO in expired air. The nasal challenge test was performed on one separate day and preceded the bronchial histamine challenge test by one day, and the first pre-exposure challenge period preceded the post-exposure challenge period by one week.

Paper V

In this study, 18 patients with bronchial asthma and nasal polyposis underwent a bronchial as well as a nasal challenge test with lysine-aspirin, and the tests were separated by at least 18 days. The nasal as well as the bronchial response were continuously evaluated throughout both tests. In the nasal challenge test, the nasal mucosa was sprayed on one occasion with lysine-aspirin, and both the nasal and bronchial response were continuously evaluated by RSM-LDF, PNIF, symptom scores and FEV1 every 10th minute for a period of 180 minutes. The bronchial challenge test was performed in step-wise increasing doses every 30th minute, and the same nasal and bronchial measurements as in the nasal challenge test every were performed every 10th minute until reaching PD20, or completing the entire test.

Paper VI

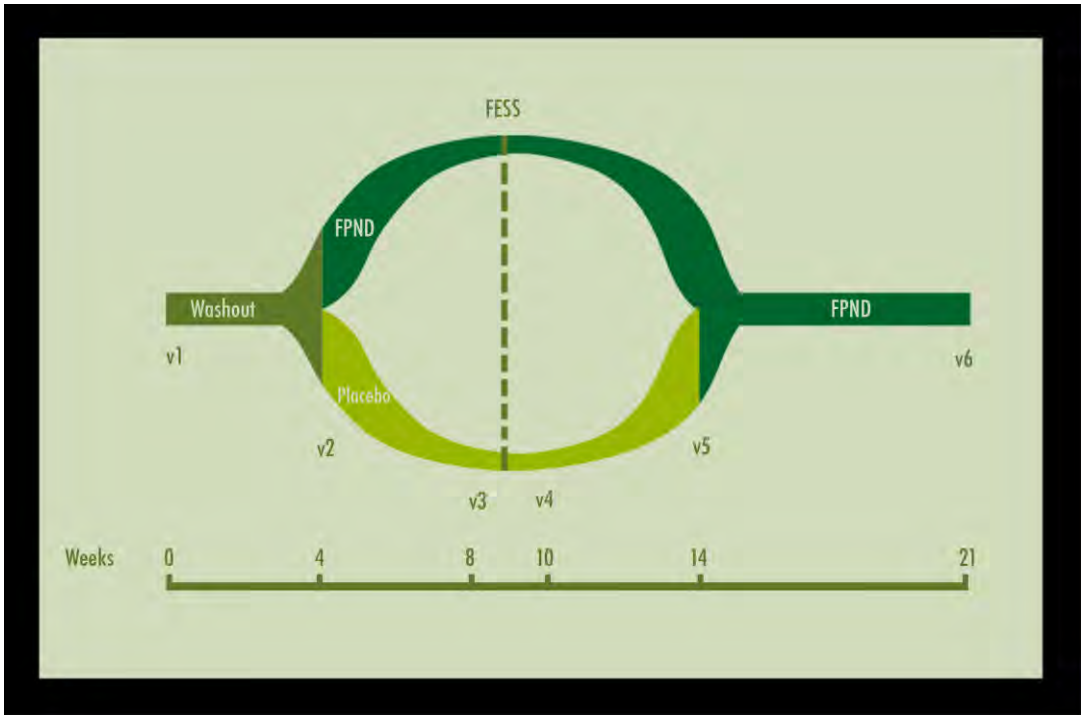


Fig. 10. Study design paper VI

This was a prospective 21 week single-centre study (Visits 1-6), conducted at the ENT and Pulmonary departments of the Karolinska University Hospital, Huddinge in order to evaluate possible bronchial and nasal benefits on treatment with FESS. A randomized, double-blind, placebo-controlled phase of 14 weeks (Visits 1-5) was also included because we also wanted to evaluate whether fluticasone propionate nasal

drops had benefits on the same parameters.

During Visit 1 the patients were evaluated by a pulmonologist and an ENT physician. Thereafter, they underwent four weeks' wash-out from nasal steroid treatment until Visit 2, where they were randomized to either FPND or placebo treatment. After four weeks of treatment and prior to surgery, the patients were examined during Visit 3. Thereafter all patients underwent FESS, and a few days later they underwent post-surgical follow-up (nasal debridement) during Visit 4. Then, they again were treated with placebo/FPND another 4 weeks in the same manner as pre-surgery until Visit 5, when they again were examined upon nasal and bronchial parameters as during Visits 1,2 and 3. After Visit 5 all patients were treated with FPND for another 7 weeks until completion of the study in Visit 6. The ordinary asthma medication was used throughout the entire study, and they filled in the daily symptom scores in the diary on nasal and bronchial parameters, as well as morning and evening PEFR.

NASAL TESTS

Single nasal pollen provocation (paper I)

First, about 24 hours before the histamine challenge test, a low test-dose of birch or timothy extract (10 000 Biological Units (BU), (ALK Sweden)) was unilaterally applied with a syringe on the mucosal surface to be studied, and with the guidance of a rhinostereometer. In the absence of severe reactions, a higher dose (100 000 BU) was then applied to the same area ten minutes later. Thereafter, the patients had to stay in the test room for at least an hour, with the aim of treating possible severe reactions.

Exposure during pollen season

Figures for airborne pollen grains of birch and timothy/ grass in Stockholm were obtained every day from the Palynological Institute at the University of Stockholm, from ten weeks before the pollen season challenge and until the study was completed (Pollensäsongen (The pollen season) 1993. Compendium, Palynologiska laboratoriet, 104 05 Stockholm, Sweden).

Swine dust exposure (papers III and IV)

In order to expose the subjects to swine dust, they helped to guide the pigs through a weighing box for three hours in a swine confinement building housing about 700-900

pigs, a procedure which causes a considerable amount of dust. On each weighing occasion, two to four subjects were exposed and dust exposure measurements were made at the same time. Measurements of *inhalable* (<10 μm) and *respirible* (<5 μm) dust and of endotoxins were made in two other subjects who were exposed on the same occasion, using the equipment described elsewhere [163, 164].

Acclimatization period and the baseline values of nasal parameters (Papers I, II, III, IV and V)

Before the test, the subject was acclimatized in the examination room for at least 30 minutes. When the position of the anteriomedial side of the inferior concha mucosa surface being studied with the rhinostereometer, was stable, acclimatization was finished. In Paper I and II that was as judged by the investigator and in Papers III, IV and V, it was defined to the time where no change in position exceeded 0.2 mm in three consecutive measurements, each separated by one minute. The values obtained became the baseline values of nasal mucosal swelling, and the corresponding values of the microcirculation, simultaneously measured by the LDF apparatus, became the corresponding microcirculatory baseline values. The position of the nasal mucosal swelling was maintained constantly under supervision of the investigator, while the LDF recordings of the microcirculation continued for 15-30 seconds.

In order to be able to compare the mean overall baseline swelling position and microcirculation measurements of the nasal mucosa between different tests, the values measured on the left side in paper II were compared to and subtracted from the corresponding values studied in paper I, and the same procedure was performed on the right side. In this way we got one positive or negative value for the right and one for the left side, after as compared to before exposure to pollen/ swine dust, and the mean value became the current baseline value.

Nasal challenge with histamine (Papers I, II, III and IV)

In order to avoid any possible interference with the nasal measurements, nasal histamine challenge tests were performed before the bronchial histamine challenge tests in Papers 1, 2 and 3. After establishing the parameters of the baseline mucosal

position and the microcirculation, the histamine challenge test took place. This was performed unilaterally, most on the right side, but if there were technical reasons (i.e.

septal deviation) the left side was used instead. After the baseline values were recorded, 0.14 ml histamine-free isotonic saline containing a phosphate buffer and 0.9 % bensylic alcohol (control) was applied to the area to be studied, on the anteriomedial part of the inferior turbinate. Thereafter, every 10th minute, immediately after recording the swelling/microcirculation, the same area was challenged with another 0.14 ml of histamine chloride in step-wise increasing concentrations. In Paper 1 these concentrations were: 0.13, 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 mg/ml (representing 0,0182, 0,035, 0.07, 0.14, 0.28, 0.56 and 1,12 mg histamine respectively). In Papers III and IV, the challenge procedure was rationalized to 0.5, 1, 2 and 4 mg/ml. The measurements of the nasal mucosal swelling, and the laser Doppler flowmetry recordings were made bilaterally in Paper I, II and V and entirely on the challenged side in Papers 2 and 3, and in all three papers these were performed 5 and 10 minutes after each saline or histamine application, up to the highest challenge concentration. An increased swelling exceeding 0.4 mm five minutes after a challenge with a solution of up to 2.0 mg/ml on the provoked side was considered as increased histamine sensitivity [165-167].

Nasal challenge with Lysine-ASA (Paper V)

Lysine-ASA was applied to the nasal mucosa using a 10 ml spray-pump (pump: Valois, France, bottle: Saint Gobain, France) with 100 µl volume for each spray.

The patients had a single nasal challenge procedure with the same volume challenged bilaterally. In order to avoid inhalation of lysine-ASA into the bronchi, the patient was instructed not to breathe through the nose during the spray procedure, and not to lean the head backwards until it was over. During apnea of both cavities the nasal challenge started unilaterally with two sprayings on the lateral wall, and immediately afterwards another two sprayings on the medial wall. Then, the patient bent his/her head forward with a piece of paper under the nose in order to avoid any bronchial inhalation and to collect the fluid during normal mouth breathing. The procedure was repeated in this way on the other side, and until there was no more lysine-ASA left to spray from the bottle. In total, the volume of the lysine-ASA was enough for about 10-16 sprayings on the nasal mucosa when spraying 18 mg (0.1M) and 36 mg (0.2 M) and 7-12 sprayings when spraying 25 mg (0.2 M).

MEASUREMENTS OF NASAL PARAMETERS

Fig. 11. Rhinostereometry, the investigating situation

The rhinostereometer (Papers I, II III, IV and V) (Fig. 10)

The nasal response was recorded by rhinostereometry. The recording device consists of a surgical microscope placed on a micrometer table. The table is attached to a frame and can be moved in three angular directions defining a three-dimensional co-ordinate system, in which the nasal cavity is placed. The patient to be examined bites on an individually-cast tooth splint fixed to the frame. The eyepiece of the microscope is equipped with a horizontal millimeter scale. The nasal cavity and the medial side of the inferior concha are viewed through the eyepiece. Since the microscope has a small depth of focus, the sharply viewed mucosal surface of the inferior concha is vertically directed and intersects the mm-scale. Small changes in position of the medial side of the inferior concha can be recorded in the plane of focus along the millimeter scale in the eyepiece as the swelling is changed. Changes in mucosal swelling exceeding +/- 0.18 mm can be detected with the equipment [49].

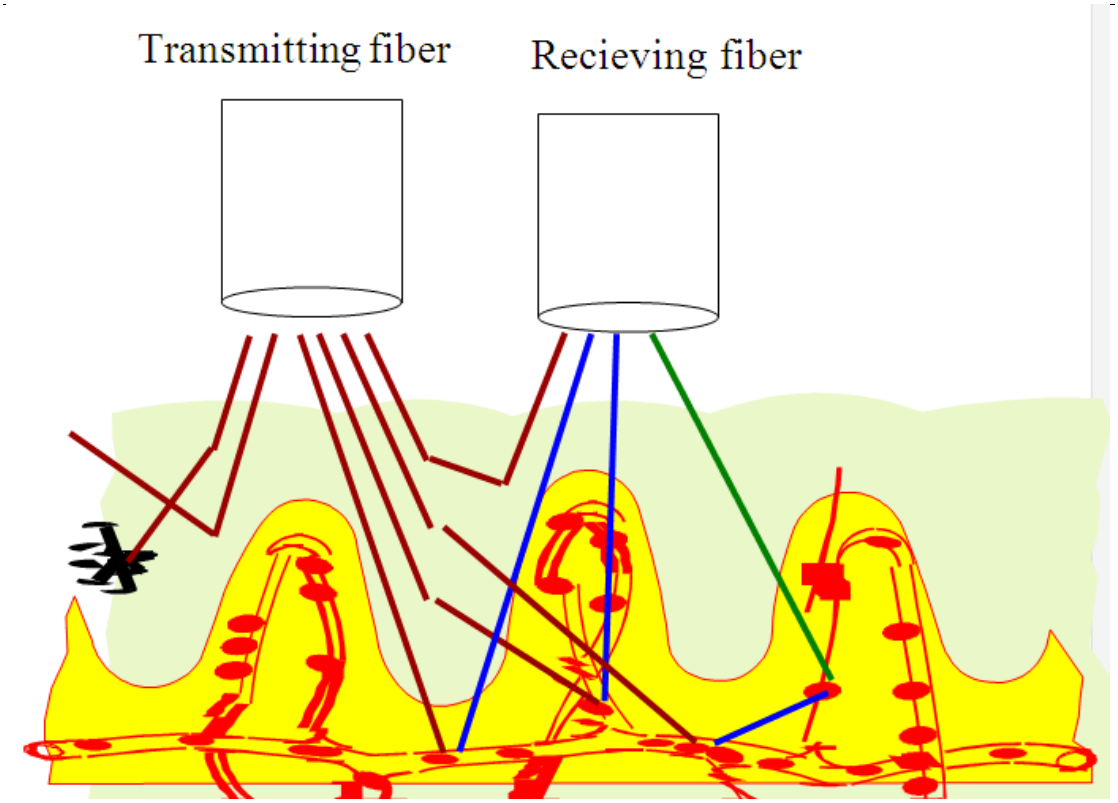


Fig. 12. The Doppler shift

The laser Doppler flowmetry apparatus measures the microcirculation in the superficial part of the nasal mucosa. Light with a wavelength of 780 nm is transmitted on to the tissue via a fiber optic probe. When the light strikes the moving blood cells, it undergoes a change in wavelength (Doppler shift), which is received by the specific fibers. A computer analyzes the data. The magnitude and frequency distribution of these changes are directly related to the number (CMBC) and mean velocity of moving blood cells in the volume measured, i.e., the blood perfusion.

VELOCITY X CMBC = PERFUSION.

The results are given in arbitrary units, and therefore the perfusion is expressed in arbitrary perfusion units (PU). PU cannot be given in ml/min/100g tissue, although there is a linear relationship between PU and ml/min/100g tissue [168]. Since the LDF apparatus is calibrated in the same way on all occasions, comparisons could be made between the measurements in each subject as well as between the subjects.

A specially designed probe with an outer diameter of 1.6 mm and a fiber separation of 0.5 mm was used, which has fibers containing efferent and afferent reflected light from the tissue. The end surface is angled at 15 degrees from the line of sight so that it stays parallel to the surface of the mucosa. The rhinostereometer, equipped with a micromanipulator to which the laser probe was attached, enabled continuous

adjustments of the laser probe end with an accuracy of 0.1mm, while keeping the measuring distance within 0.3 mm, in accordance with the criteria for precision in all three dimensions [51]. This equipment permits simultaneous recordings of changes in the congestion and the microcirculation of the same area of the nasal mucosa [50, 51]. (Fig. 2, 11, 13)

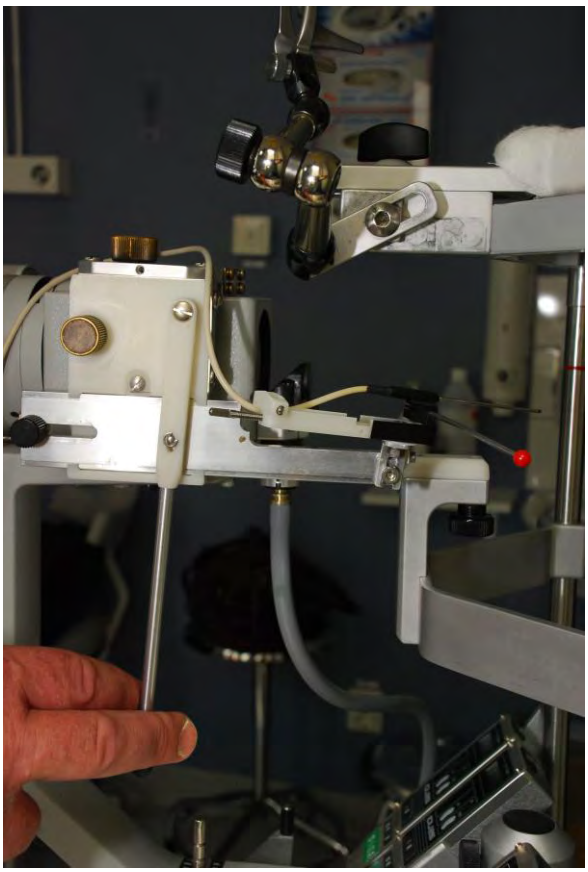


Fig. 13 The laser Doppler flowmetry apparatus

The measuring depth of the laser Doppler flowmeter is affected by the type of tissue, the wavelength, and fiber separation, and cannot be exactly determined. In human skin, when using a wavelength of 780 nm, the measuring depth is estimated 0.5-1 mm (19), and in the nasal mucosa it has been estimated to be at least 1 mm [169].

Nasal air flow (PNIF) (Papers V and VI) (Fig. 1.)

An In-check™ Portable Inspiratory Flow- meter (Clemont Clark, England) was used in order to measure the nasal inspiratory airflow. First, the patient was instructed how to use the equipment and when the investigator judged the technique to be adequate, the best value from at least three inhalations was used establishing the baseline value.

During challenge, PNIF was recorded every 10th minute, and the best value of two exhalations was registered.

Nasal lavage (Papers III and IV)

We performed nasal lavage with 0.9% NaCl at 37°C (12), but with minor modifications (5). The subjects were seated with their necks flexed backwards to about 45 degrees, and 5 ml was then instilled into one nostril, using a syringe without a needle. After 10 seconds, they bent forward and the liquid ran out into a plastic basin.

The volumes of the lavage from each nostril were measured and centrifuged at 200 g for 10 min at 4° C and the supernatant frozen at –70° C, pending the analyses. The pellet was resuspended in 0.9% NaCl with 0.1 % human serum albumin.

The cells were counted in a Bürker chamber and the cell concentration in the lavage fluid recovered was calculated. The concentration of human serum albumin in the nasal lavage supernatant was measured, using inhibition ELISA (enzyme-linked immunosorbent assay) [125]. The lower detection limit of the assay was 25 ng/l.

Interleukin-8 was measured in duplicate with an ELISA method using commercially available antibody pairs (capture antibody (MAB208), detection antibody (BAF208) and standard (208-IL- 101), R&D Systems Europe, Abingdon, U.K.). The lower detection limit of the assay was 25 ng l^{-1}.

Butanol threshold test of the olfactory function (Paper VI)

Prior to a decongestant during Visits 2, 3, 5 and 6, the olfactory threshold was determined using butanol in dilutions ranging from 0.000008% to 4%. The olfactory threshold was identified when the subject was able to distinguish the same butanol concentration from a blank control on five consecutive attempts [53, 54]. The grading of this test is: normal olfactory function when the threshold is 7-14, hyposmia 3-6, and anosmia 0-2.

Nasal endoscopy (Paper VI) (Fig. 3.)

A nasal endoscopy was performed by otorhinolaryngologists on all visits and was scheduled after PNIF and the butanol threshold test. The nasal cavity was decongested prior to endoscopy with Lidocaine hydrochloride + Nafazoline, 34 mg/ml + 0.17 mg/ml (colored). The nasal polyp size was scored on a 0-3 scale [53]: (0= no polyps, 1= polyps in the middle meatus, not reaching below the inferior border of middle turbinate,

2= polyps reaching below the inferior border of the middle turbinate, but not the lower border of the inferior turbinate, 3= polyps reaching lower than the inferior border of the inferior turbinate and/or medial to the middle turbinate.).

Nasal symptom scores

Baseline nasal symptom scores (Papers II, IV and V)

In Paper II the baseline nasal symptom scores before starting the nasal challenge test were estimated with visual analogue scales (VAS), each a 100 mm solitary line on a separate paper. A separate scale for each type of symptom was used to estimate the severity of nasal symptoms on that day. The symptoms were: *blockage*– from free to totally obstructed nose, *rhinorrhoea* – from no rhinorrhoea to intolerable wet nose, sneezing – from no sneezing to intolerable and running. In Paper IV the subjects evaluated their degree of nasal patency only, using the same VAS scales as in Paper II.
In Paper V the patients estimated the baseline nasal symptoms using a score number between 0 and 10. The symptoms were: *stuffiness*- from free (0) to totally obstructed nose (10), and *rhinorrhoea* - from dry nose (0) to an intolerably runny nose (10).

Nasal symptom scores during nasal challenge (Paper V)

In Paper V, the patients continued to estimate the symptoms of patency and rhinorrhoea every 10th minute in the same manner as the challenge test started. After finishing the test, the change in symptoms in relation to the baseline was calculated.

Counting the number of sneezes throughout histamine challenge (Papers I1 and V)

For each patient, the total number of sneezes was counted throughout the nasal histamine challenge test.

3.2.1.1 Diary cards nasal parameters (Paper VI)

Nasal symptoms scores

Patients graded the symptoms of nasal congestion and rhinorrhoea, respectively, on a 0-3 scale (0 = no symptoms, 1 = mild symptoms/ tolerable, 2 = moderate symptoms/ still tolerable, and 3= severe symptoms/ affects daily activity). The sense of smell was also graded on a 0-3 scale (0 = normal, 1 = mild reduction, 2 = moderate reduction, 3 =

absent sense of smell). Adherence to the study treatment was also reported in the nasal symptom score diary by the patients.

BRONCHIAL TESTS

3.2.1.2 Bronchial challenge with histamine (Papers I, II, III and VI)

A jet nebulizer was used (Ailos, Medicinsk Teknik AB, Karlstad, Sweden) for the bronchial histamine challenge, with inhalation of first saline and then histamine by tidal breathing for 30 seconds in Papers 1 and 2, and in Paper 5 this was instead conducted by the use of a dosimeter-controlled jet nebulizer (Spira Elektro 2™, Respiratory Care Center Ltd, Hemeenlinna, Finland) (Fig. 14).

Fig. 14. The jet nebulizer
Inhalation of histamine and aspirin (paper III and V) was performed with a dosimeter-controlled jet nebulizer

In all studies the aerosol was inhaled through a mouthpiece, while using a nose clip In Papers I, II and III we used PC_{20}-PEF for evaluation of the histamine sensitivity, and in Paper V and VI we used PD_{20} FEV1 for evaluation of the lysine-aspirin and histamine sensitivity respectively.

For determining the baseline value, in all studies the best of three PEF (Papers I, II, III) /FEV1 (Paper V,VI) measurements was registered. Then, the subjects inhaled 0.14 ml of histamine-free phosphate buffer, containing 0.9% bensylic alcohol, and the subjects continued the test by inhaling increasing concentrations of histamine, also containing 0.9 % bensylic alcohol in the histamine solutions. The doses were doubled from 0.13 mg/ml to a maximum of 16 mg/ml. The measurements were performed three minutes after inhaling the buffer solution as well as the histamine

concentrations, and throughout the challenge test, the better of two values was registered.

In Papers I, II and III, the test were stopped when the reduction in PC_{20}-PEF was 20 % or more of the initial value. The histamine concentration that provoked a 20% fall in PC_{20}-PEF was calculated by linear interpolation of the last two points on the non-cumulative concentration-response curve (PC_{20}-PEF). For the statistical analyses of correlations between nasal and bronchial histamine sensitivity in paper II, PC_{20}PEF values were divided into 8 classes: values of: =16 mg/mL, 8 – 15.9 mg/mL, 4 – 7.9 mg/mL, 2 – 3.9 mg/mL, 1 – 1.9 mg/mL, 0.5 – 0.99 mg/mL, 0.25 - 0.49 mg/mL and < 0.25 mg/mL.

Also in Paper VI, inhalation of diluent was followed by incremental doses of histamine phosphate (prepared at Norrlands University Hospital Pharmacy, Umeå, Sweden) administered at three minute intervals. Three concentrations (1, 8 and 64 mg/mL) and 2, 4 and 8 breaths were used to create increasing doses (range; 14 -3520 μg). The test was terminated when FEV_1 had fallen at least 20% from the post-diluent baseline, or the maximum cumulative dose of histamine had been reached (7027 μg). After the challenge the patient was observed until FEV_1 had returned to within 90% of baseline. The histamine $PD_{20}FEV_1$ values were calculated from the log-dose response curves by linear interpolation [121]

Bronchial challenge with lysine-aspirin (Paper V)

A bronchial challenge was performed using the same dosimeter controlled jet-nebulizer for lysine-aspirin inhalation as in Paper VI (Fig. 14).

The bronchial provocation started by inhaling nine breaths of NaCl, with measurements of FEV1 10 and 20 minutes after inhalation. Then, starting 20 minutes after NaCl inhalation, lysine-aspirin was inhaled creating increasing cumulative doses every 30th minute. The lysine- aspirin schedule formed an approximately half log increase in a cumulative ASA dose (Table 2). 10, 20 and 30 minutes after inhalations, first nasal and then spirometry measurements were performed after the measurements of nasal swelling and microcirculation, as described above.

The challenge was stopped when FEV_1 had decreased by 20 % or more compared to FEV_1 20 minutes post diluent, or when the maximum dose was reached. When FEV_1

reached a decrease of 20% or more throughout the challenge test, PD_{20} was then calculated as described in "Statistics". Bronchoconstriction was reversed immediately

by inhaling 5 mg Salbutamol (Ventoline®, Glaxo Wellcome, England) and 0.5 mg Ipratropiumbromid (Atrovent®, Boehringer Ingelheim, Germany).

Measurements of lung function (Papers I, II, III, and V) (Fig. 4.)

A Wright peak flow meter was used for measuring the lung function and further calculations of PC_{20}-PEF in Papers I, II and III, a Vitaloraph ALPHA II® spirometer (Förbandsmaterial AB, Partille, Sweden) was used for this purpose instead. In Paper V and VI, FEV_1 was measured with a Spirolab spirometer (Medical International Research, Holland), and in Paper V the lung function was measured by a Spirolab®, MIR, Italy, spirometer. All the measurements were performed according to the current statement of the American Thoracic Society.

Diary cards concerning bronchial parameters (Paper VI)

From screening during Visit 1 to the end of the study in Visit 6 (except between Visits 3-4), patients had diary cards on a daily basis in order to register PEFR, asthma symptom scores, and as needed β2-agonists for asthma. We calculated the mean daily symptom scores, the mean daily PEFR, and the mean number of inhalations with short acting β2-agonists from the diary cards the last 7 days prior to Visit 2 (baseline recordings) and compared to scores of the last 7 days prior to Visits 3, 5 and 6.

a). PEFR *(Fig. 5, 15)*

Fig. 15. PEFR, investigation situation

The morning and evening PEFR were measured (Personal Best®, Health Scan Products Inc, USA) and the results were filled in at the time. The mean daily PEFR were then calculated.

b). Asthma symptoms score

On a separate page patients were asked about their asthma symptoms: shortness of breath and cough. The symptoms were graded on a 0-3 scale (0 = no symptoms, 1 = mild symptoms-/tolerable, 2 = moderate symptoms/still tolerable, and 3 = severe symptoms/affects daily activity).

c). As-needed β2-agonists for asthma

Patients were instructed to use short-acting β2-agonists for as-needed asthma medication, and to register the number of inhalations in their diary. When completing

the statistics, the frequency of inhalations was graded as follows: 0 inhalations= 0 points, 1-2 inhalations = 1,5 points, 3-5 inhalations = 4 points, >5 inhalations = 5 points

3.2.2 FESS surgery (Paper VI)

The majority of FESS were performed by one out of a total of six ENT surgeons performing surgery in this study. All patients were under general anesthesia. Local anaesthesia with Lidocaine-hydrochloride 10 mg/ml epinephrine 5 microg /ml was also used to minimize bleeding and improve visibility. The procedure was tailored to the extent of the disease as indicated by clinical and CT scan findings, but usually included the removal of polyps, uncinectomy, anterior ethmoidectomy and exploration of the posterior ethmoids. If the posterior cells were involved, surgery was continued posteriorly with posterior ethmoidectomy and, in some cases sphenoidotomy. The ostium to the maxillary sinus was enlarged and diseased mucosa from the fronto-nasal recess was removed. If there was a pneumatized concha bullosa of the middle turbinate, the lateral mucosa and bone were usually removed to decompress the ostieomeatal complex. For subjects who had previously undergone FESS, the extent of surgery depended on clinical findings, and in some cases a simple removal of polyps was sufficient.

3.3 STATISTICS

Students' T-test for paired groups was used in Paper I for statistical analyses of nasal swelling and for a comparison of dust exposure and lung function variables in Papers 1 and 2.

The Fisher exact test was used to analyze the relation between upper and lower airways reactivity in Paper I.

Friedman´s ANOVA, repeated measurements were calculated by a statistician at the Department of Medicine at Karolinska Hospital, Huddinge in Paper III.

Wilcoxon´s matched paired test was used for a non-parametric statistical analysis within the groups.

Mann-Whitney U was used for a non-parametric statistical analysis between groups, calculated by the authors in Paper II, at the Department of Mathematical Statistics at the University of Stockholm in Paper IV, and by Clinfile AB in Paper VI.

Spearman´s rank correlation test was used for correlation calculations in Papers I, II, III, IV and VI

Mixed (effect) Models was used for a statistical analysis of clinical parameters within and between groups in Paper V, as calculated by a statistician at the Lime Group at Karolinska Institutet, Stockholm. Mixed Models is a regresssion analysis method, a development of ANOVA, repeated measurements, with the advantage of performing more flexible estimations than ANOVA. This results in benefits when comparing different groups with different variability, i.e. healthy subjects vs. patients or ATA vs. AIA patients. The measurements of the control group are often more stable with a lower variability than in the patient group, which might be a statistical problem when comparing groups. In this sense, Mixed Models is meant to handle with these differences in variability better than ANOVA.

4 RESULTS AND COMMENTS

4.1 PAPERS I AND II

RESULTS

- Out of pollen season, mean swelling, measured by RSM, throughout the histamine challenge test was increased compared to the baseline after an application of histamine 0.25 mg/ml ($p<0.05$), and it was greater than in a reference group of healthy volunteers five minutes after challenge with histamine 2 mg/ml ($p<0.01$) **(**Fig. 16)

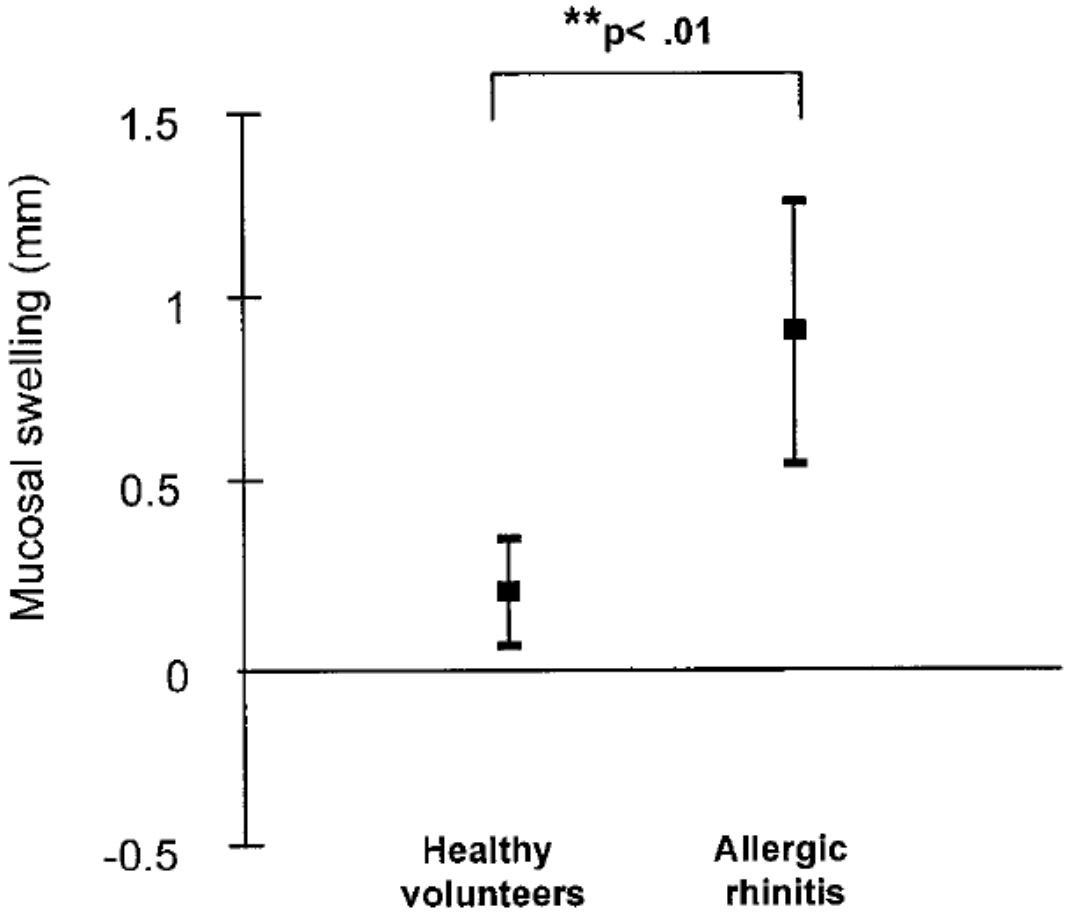


Fig. 16. Nasal mucosal swelling out of pollen season
Mean values of nasal mucosal swelling with 95% confidence intervals.

- The histamine sensitivity as measured by RSM was increased in the same way over the seasons (Fig. 17), although the nasal symptoms increased after pollen exposure.

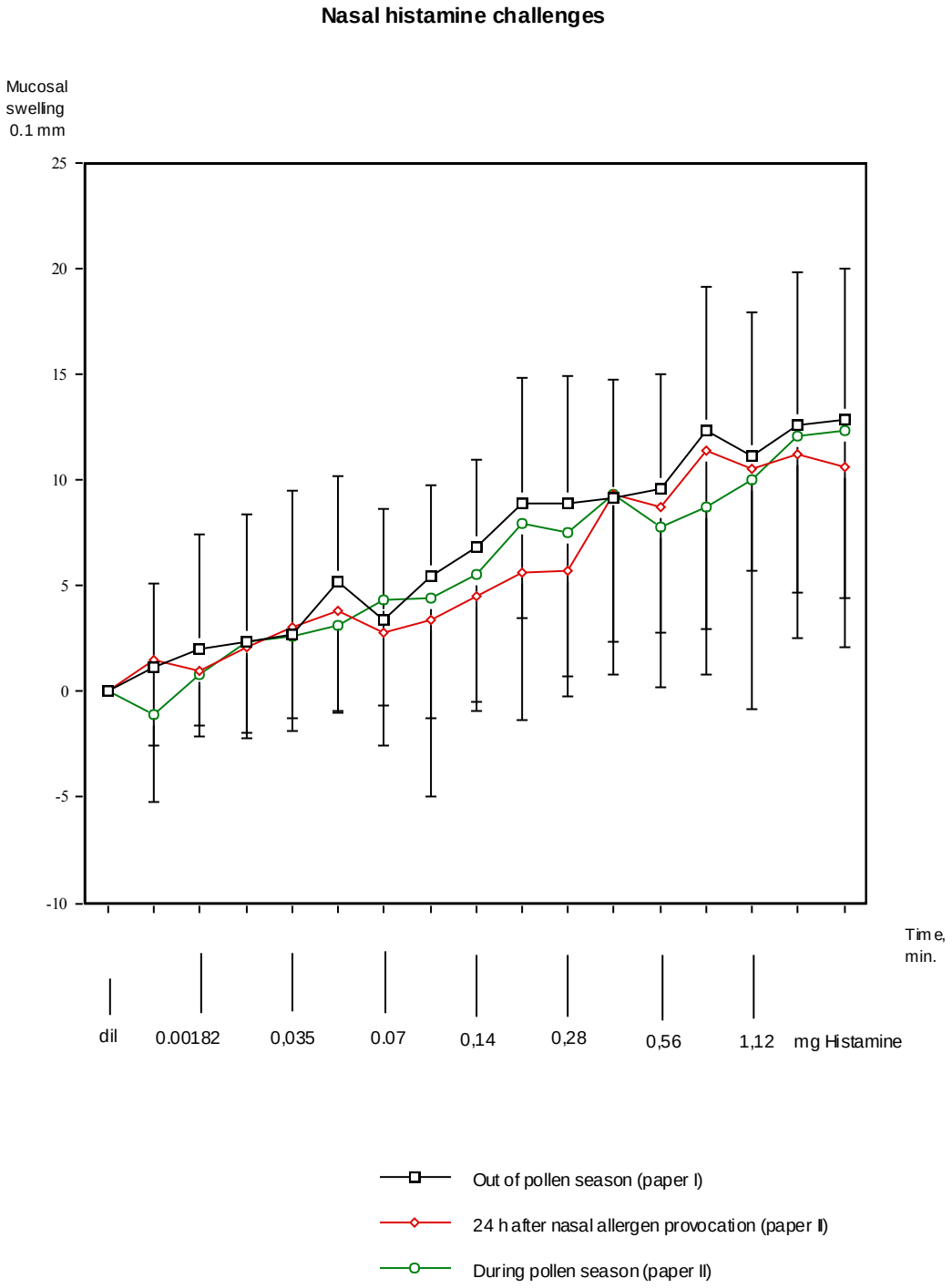


Fig. 17.
Nasal mucosal swelling during histamine challenge test out of pollen season, after nasal allergen provocation and during pollen season ($p<0.05$, Wilcoxon's signed rank test).

- The bronchial responsiveness was also unchanged during the pollen season, when using 1.12 mg histamine (0,14 ml 8 mg/ml) as a cut-off level. We found no intra-individual correlation between nasal mucosal swelling and PC20 during or out of pollen season.

- The histamine sensitivity as measured by the scores concerning sneezing increased significantly at group level 24 hours after the nasal pollen challenge ($p<0.05$). During the pollen season we found a negative correlation between the number of sneezes following the histamine challenge and PC_{20}-PEF ($p= 0.0071$, $R= -0.74$). However, the negative data during the season may be due to the small number of patients.

COMMENTS

Provocation tests of the nose and bronchi are tools for exploring the nonspecific reactivity of the nasal and bronchial mucosa [1]. However, while nonspecific bronchial provocation is widely used in clinical practice and tests are standardized enough, the clinical usefulness of nasal tests still requires more accurate evaluation, since the methods for studying nonspecific nasal reactivity are not specific, sensitive and standardized enough [1]. Therefore, we wanted to evaluate RSM as a method for a nasal challenge and the detection of nasal hyperresponsiveness in pollen allergy patients. As we wanted to study both the upper and lower airways in the same individuals, we chose the same agent for the nasal and bronchial challenge tests despite the fact that histamine more commonly than methacholine initiates side effects such as itching, sore throat and headache throughout bronchial challenge tests. Histamine is the most potent mediator in vasomotor responses, and acts both directly at the mucosal level and through nervous reflexes on vessels and glands [120, 170].. In the current study (Paper II), we found that significant swelling occurred already at low histamine concentrations out of pollen season, and the overall response was roughly similar to that found in patients with perennial, non-allergic rhinitis, where the same method was used [165, 166, 171, 172]. The increased histamine sensitivity out of pollen season demonstrated in our study, can be seen as a sign of "a minimal persistent inflammation" [173]. This may be the reason that a single nasal pollen provocation as well as seasonal pollen exposure did not change nasal histamine

sensitivity compared to out of season, as measured by an increase in mucosal swelling. Conflicting evidence regarding this issue has been previously published.

In a previous study of ten pollen allergy patients out of season, where the nasal mucosal swelling, measured by RSM, was also increased as compared to the case in 10 healthy subjects after a single challenge with 0.14 ml 1 mg/ml histamine [174].

A difference in histamine sensitivity between pollen allergy patients and healthy subjects out of pollen season has also been shown previously when using acoustic rhinometry as a detection method [46]. However, in the same study there was an increased histamine sensitivity in the pollen allergic group after a nasal pollen provocation. These results support the view that years of seasonal allergen exposure may induce chronic inflammatory changes in the airway mucosa.

On the other hand, Hellgren and co-workers found no significant differences in nasal histamine sensitivity between pollen allergy patients and healthy subjects out of pollen season, when challenging with very low doses of histamine and the nasal response was evaluated by symptom scores, anterior rhinomanometry, acoustic rhinometry, nasal peak expiratory (PNEF) and inspiratory (PNIF) flow [48]. Interestingly, when using the highest concentration of histamine (1 mg/ml), the allergic group tended to respond stronger to histamine, as measured by PNIF indicating that the doses were too low to detect differences between groups,

An important feature of non-specific nasal reactivity is that the etiology is not fully known, and that it may be elicited by various mechanisms. This clinical heterogeneity of rhinitis demands an individually-tailored provocation test, taking into consideration the current symptoms of the specific individual to be challenged [1]. An example of this is the individual variability in the frequency of histamine-induced sneezing registered in this study. However, on a group level, these increased after pollen exposure, which is in line with previous findings [175], where measurements of sneezes throughout the histamine challenge reflected the increase in allergic nasal symptoms, and also with a correlation to the bronchial histamine sensitivity, in line with the results of our study (Paper II). We think that this is not in conflict with our RSM data on the histamine challenge; the reason being that sneezing is a neurogenically-histamine mediated response whereas mucosal swelling probably is due to a direct histamine effect on the vasculature. Measurements of bronchial responsiveness reflect the sensitivity of the airways to trigger factors that can cause

asthma symptoms, and the results are usually expressed as the provocative concentration/dose of the agonist causing a given fall (often 20%)
in FEV1 or PEF. No histamine threshold concentration has been generally accepted as defining bronchial hyperresponsiveness. PC_{20}-PEF -values ranging from 4 to 16 mg have been reported as threshold values in various studies [176, 177]. We used 8 mg/ml as a suitable threshold value, because two of the three patients who had previously reported mild episodes of asthma had a PC_{20}-PEF of less than 4 mg/ml during the pollen season. In other studies of the bronchial response to non-specific irritating agents in hay fever patients, a seasonal variation with an increase after pollen exposure has often been shown [147-149]. Therefore, it is possible that the patient group in this study was too small to detect seasonal differences in bronchial responsiveness, and perhaps $FEV_{1.0}$ would be a more sensitive method than PEF for detecting seasonal differences in PC_{20}.

4.2 PAPERS III AND IV

RESULTS

Nasal histamine tests

- When NH was performed before NAL (Group 1), the histamine sensitivity was increased afterwards as compared to before exposure to swine dust with an increase in swelling ($p=0.012$) (Fig. 18), in nasal blockage before starting the histamine challenge test ($p=0.017$), and a decrease in CMBC ($p<0.001$).

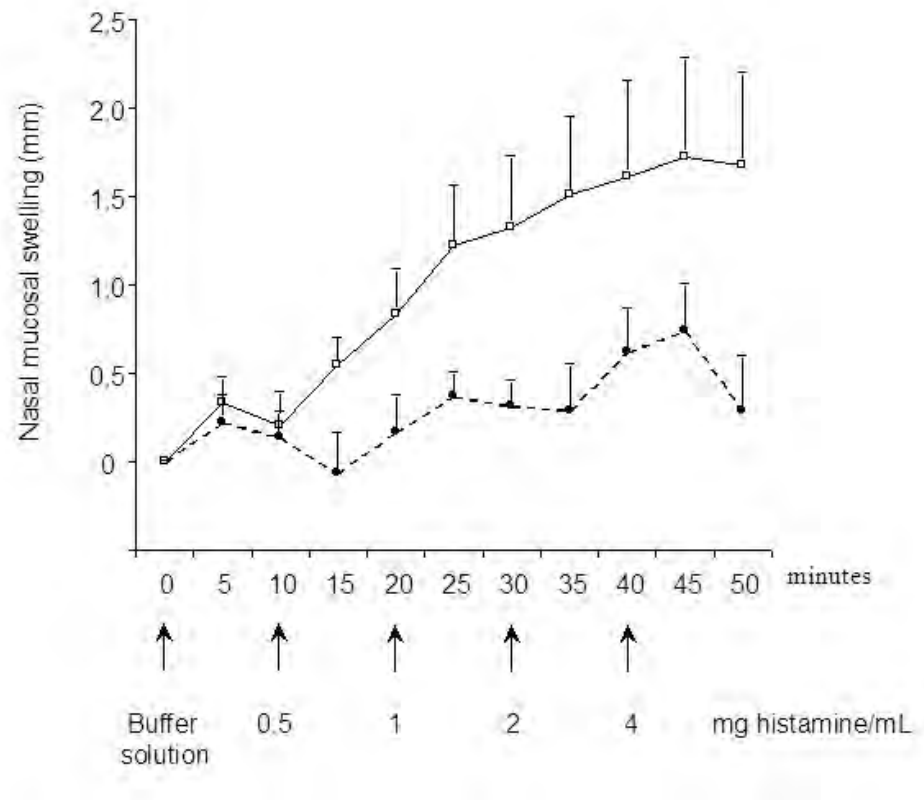


Fig. 18. Nasal mucosal swelling during histamine challenge test before and after swine dust exposure in Group 1 (NH before NAL). (Mean values +/- SEM). *The filled symbols indicate before exposure to swine dust (■) and the empty (□) after exposure to swine dust. ($p<0.05$, Wilcoxon's signed rank test).*

- The levels of cells ($p<0.01$), albumin ($p<0.01$), and Il-8 ($p<0.05$) in NAL were also increased. Further, we found a significant inverse correlation between the Δ- albumin levels in nasal lavage and the baseline Δ-CMBC ($R= -0.95$, $p=0.018$).
- When NAL was performed before NH (Group 2), the histamine sensitivity was not increased as swine dust exposure did not affect the histamine-induced swelling (Fig. 19**)**.

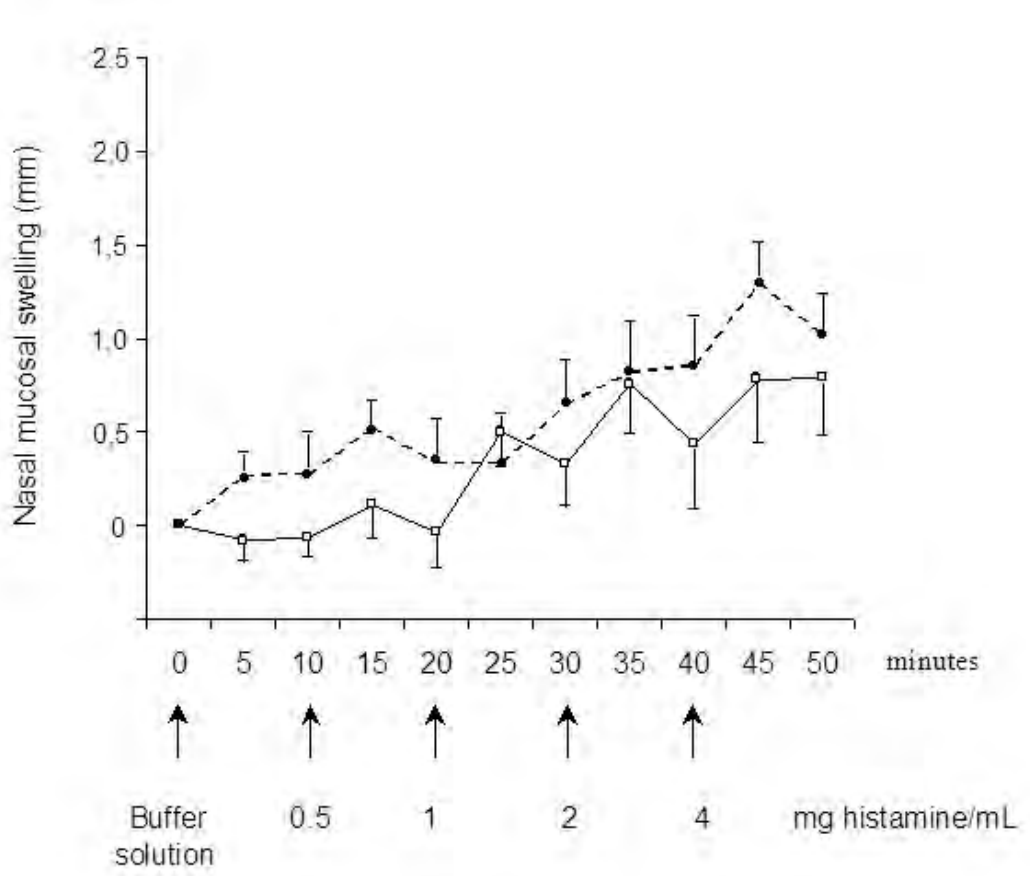


Fig. 19. Nasal mucosal swelling during histamine challenge test before and after swine dust exposure in Group 2 (NH after NAL). (Mean values +/- SEM).

The filled symbols indicate before exposure to swine dust (■) and the empty (□) after exposure to swine dust.

- Instead, there was a decrease in perfusion throughout the histamine challenge as compared to pre-exposure (p=0.0002)(Fig. 20).

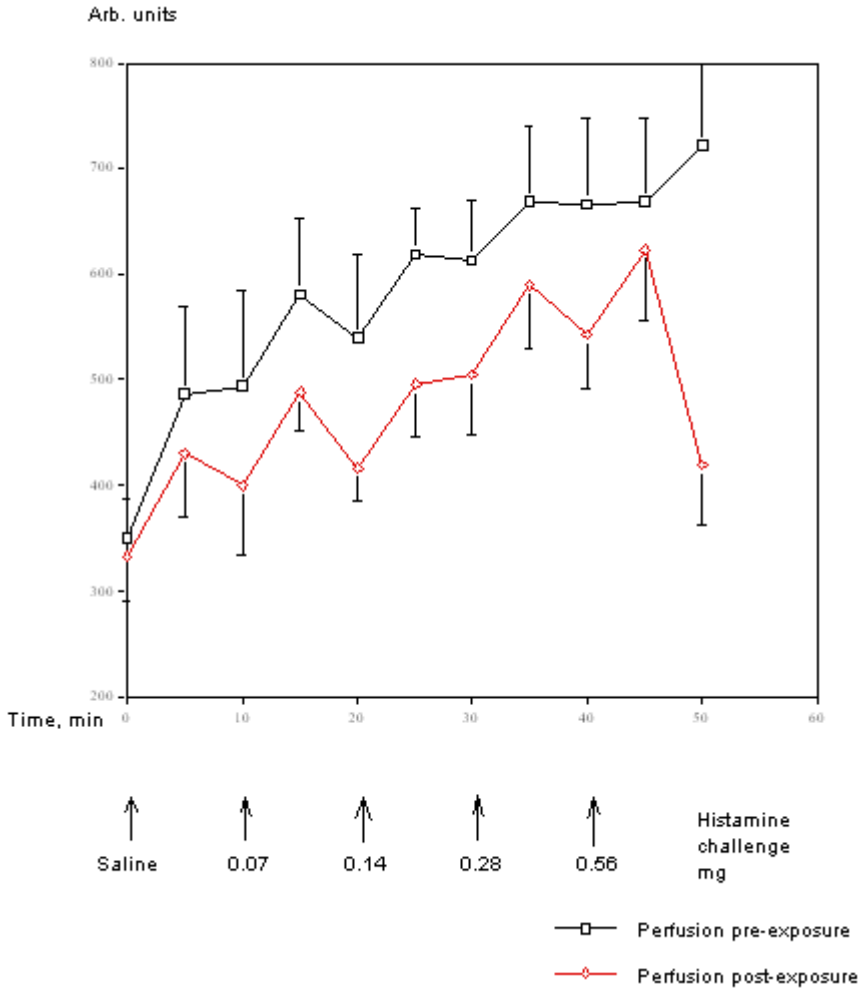


Fig. 20. The effects of exposure to swine dust on perfusion during histamine challenge test in Group 2 (histamine challenge *after* nasal lavage). *(*p<0.0002, Wilcoxon´s signed rank test). This was not true of Group 1.*

Bronchial histamine tests

- Compared with the baseline, FEV_1 and FVC were significantly lower at three and 24 hours following exposure ($p < 0.002$), and PEF was significantly lower compared to baseline three hours after exposure ($p<0.005$). PC_{20} PEF, decreased significantly 24 hours after as compared to before exposure ($p<0.045$) (Fig. 21)

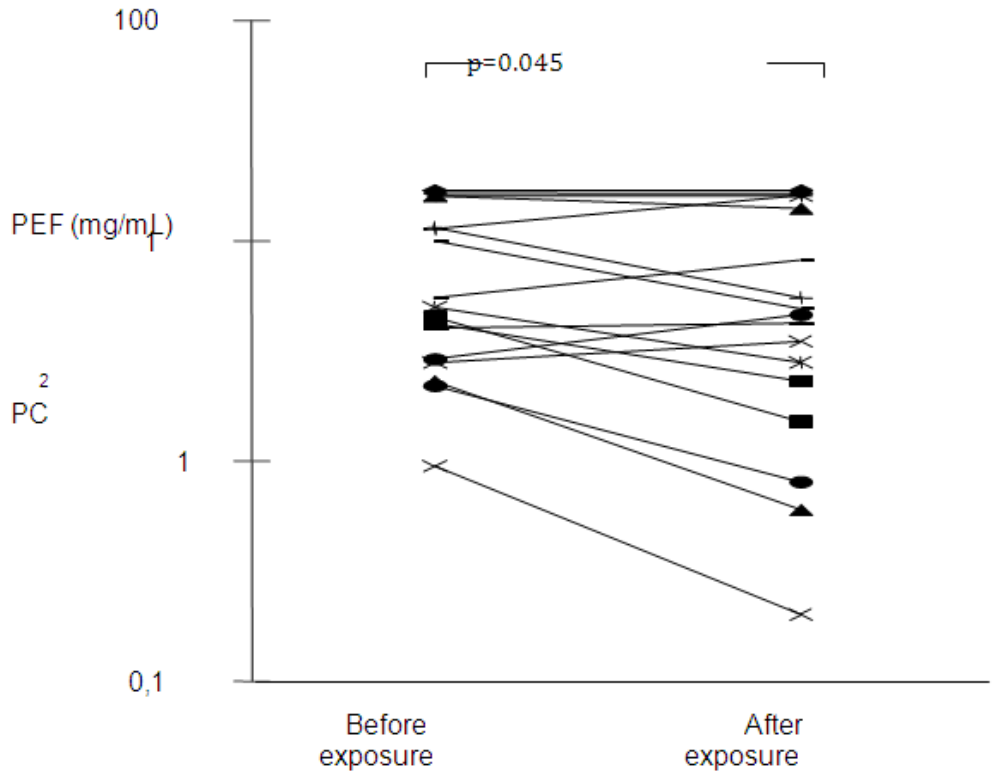


Fig. 21 PC_{20}PEF before and after swine dust exposure

There was a significant decrease in PC_{20}PEF after as compared to before exposure (p= 0,045, Wilcoxons matched pair test).

- We found no significant correlation between PC_{20}PEF and nasal reactivity in Group 1 (NH before NAL) before or after dust exposure.

COMMENTS

In this study, we evaluated the usefulness of short time swine dust exposure as a model for upper and lower airway inflammation in healthy subjects, and compared these results with those of pollen allergy patients before and after exposure to pollen in Papers I and II. Therefore, these subjects were also challenged with histamine chloride in the nose and bronchi before as well as after exposure to swine dust, with measurements of nasal mucosal swelling, nasal symptom scores and PC20-PEF. However, in contrast to the first study (Papers I and II), in Paper IV we also used a laser Doppler flowmeter attached to the rhinostereometer apparatus allowing simultaneous measurements of the nasal mucosal swelling with increased blood flow in the capacitant vessels deep in the nasal mucosa, as well as of the

microcirculation in the superficial vessels at a certain area of the mucosa, i.e. on the anteriomedial part of the inferior turbinate.

Swine farmers have increased frequency of airway symptoms [178], and analyses of broncho-alveolar lavage (BAL) have detected signs of a permanent airway inflammation in this group [179, 180]. It has previously been shown that three hours of intense exposure to swine dust by weighing pigs in a swine house is a useful model for inducing a massive inflammation, and in our study we confirmed previous results [125, 127]), such as increased levels of neutrophil cells, IL-8 (which is an important chemo-attractant for neutrophils [181], and albumin in nasal lavage. A marked increase in the sensitivity to metacholine in the bronchi has also been found [182], which persists above pre-exposure levels for up to one week after three hours of exposure to swine dust [130].

Therefore, we have verified that the subjects in our study actually had an ongoing inflammation when our measurements took place.

Although the symptoms of nasal blockage increased after swine dust exposure, neither the baseline swelling nor the baseline perfusion increased, as we expected. In comparison, the baseline swelling did not increase in the pollen allergic group after pollen exposure despite increased symptoms of nasal blockage (Paper II). Obviously, increased nasal congestion, which is a sensation, is not equal to increased measurements of nasal mucosal swelling and perfusion at a certain area of the inferior turbinate. However in a previous study, an obstruction in airflow of the nasal cavity was detected by acoustic rhinometry after exposure to swine dust [127]. The main difference between RSM and acoustic rhinometry is that the former measures direct changes in swelling at a certain area on the inferior turbinate, and the latter measures internal nasal luminal volume, and the minimum cross-sectional area, and this is based on reflected sound waves. Therefore, these tests are not comparable [183]. Throughout the histamine challenge, we found an increase in swelling (and a decrease in CMBC) under inflammatory conditions as compared to non-inflammatory conditions, and that is in contrast to the situation in the study of the pollen allergy patients, where exposure to pollen did not induce such changes. This result is in accordance with our previous deduction that patients with allergy suffer from "minimal persistent inflammation" with a long-lasting increased histamine reactivity out of pollen season as measured by RSM. Interestingly, the results of the bronchial histamine challenge test are similar to those of

the nasal histamine challenge tests with measurements of swelling, with increased bronchial histamine sensitivity under inflammatory conditions in the group of healthy subjects, but not in the group of allergy patients, where BHR is similarly increased outside of as well as throughout pollen season. Today, we do not pay so much attention to the NO-measurements performed in Paper III, because when the experimental part of this study was performed, this technique was new and perhaps premature, and more convenient technology is used today for these measurements.

Another aim of this study was to evaluate RSM-LDF as a method of detecting nasal hyperreactivity throughout the histamine challenge, and we found microcirculatory effects both of swine dust exposure per se, and of NAL. Interestingly, the changes in baseline CMBC after exposure correlated with the corresponding changes in albumin in Group 1. We chose albumin as the detector of vascular leakage although it leaks continuously through the vascular endothelium due to its relatively low molecular size, with detectable levels also in baseline samples under non-inflammatory conditions (Paper III and IV). Vesterberg et al. showed that Alpha2-macroglobulin, of a greater molecular size, is a better marker than albumin of plasma extravasation in the airway mucosa after exposure to swine dust, with a correlation with BAL- fluid neutrophilia [126]. However, in the same study, a correlation was found between the levels of Δ-albumin and Δ-alpha2-macroglobulin in BAL, Albumin thus seems to be an acceptable marker of vascular leakage, and the presence of higher concentrations of albumin in the NAL reflects an increase in vascular leakage of the nasal mucosa. These results strengthen the hypothesis that an interstitial edema due to vascular leakage can be detected non-invasively by a reduction in CMBC [169]. However, to confirm this hypothesis, it demands further studies designed with the purpose of answering this question.

This study (Papers III and IV) was also designed in order to also evaluate whether NAL would affect the outcome of the clinical and subjective nasal measurements, and therefore the patients were divided into two groups, with the opposite order of measurements of RSM-LDF/symptom scores and nasal lavage. We found that NAL had a significant effect on nasal mucosal swelling, microcirculation as well as symptoms, and therefore these investigations should be separated in time. An increasing number of papers on the efficacy of nasal irrigation in inflammatory disorders of the nasal mucosa have been published [184, 185], and our findings are

interesting in this regard. In nasal irrigation, the volume of saline is much higher, and the effects probably greater, and that implies that nasal irrigation may be a simple and cheap method for the anti-inflammatory treatment of the nasal mucosa.

4.3 PAPER V

RESULTS

Bronchial challenge

- The bronchial challenge test was positive in ten of the 18 patients, with a geometric mean PD20 value of 43,4 μmol aspirin in that group. In one subject the bronchial provocation test was negative, but due to the history, the patient later had an oral aspirin provocation test, which was judged as positive. Therefore, 11 subjects were included in the AIA group and seven in the ATA group.
- In the AIA groups, CMBC decreased compared to after the saline challenge within the time span between 60 minutes before until 60 minutes after they reached PD20, in total nine measurements (p=0.041) (Fig. 22)

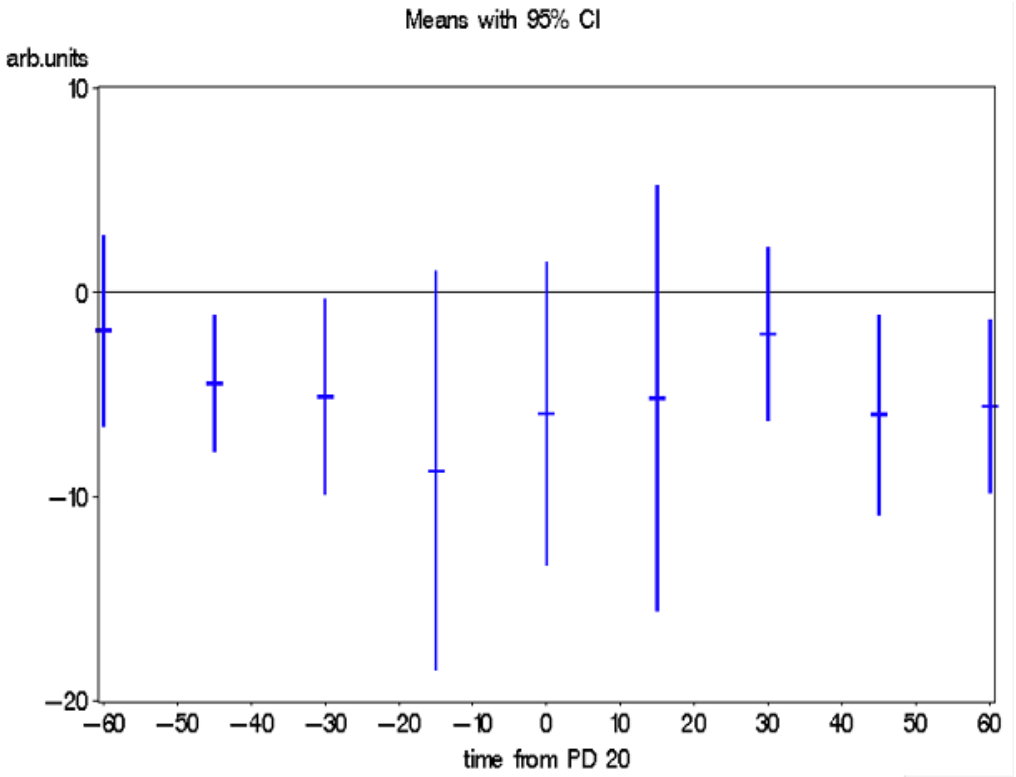


Fig. 22 CMBC (concentration of moving blood cells) in relation to PD_{20} in the AIA group throughout the bronchial challenge test with Lysine-aspirin**.**

Nasal challenge

- The perfusion was significantly increased as compared to the ATA group in the interval 10-180 minutes after challenge (p=0.033)(Fig. 23).

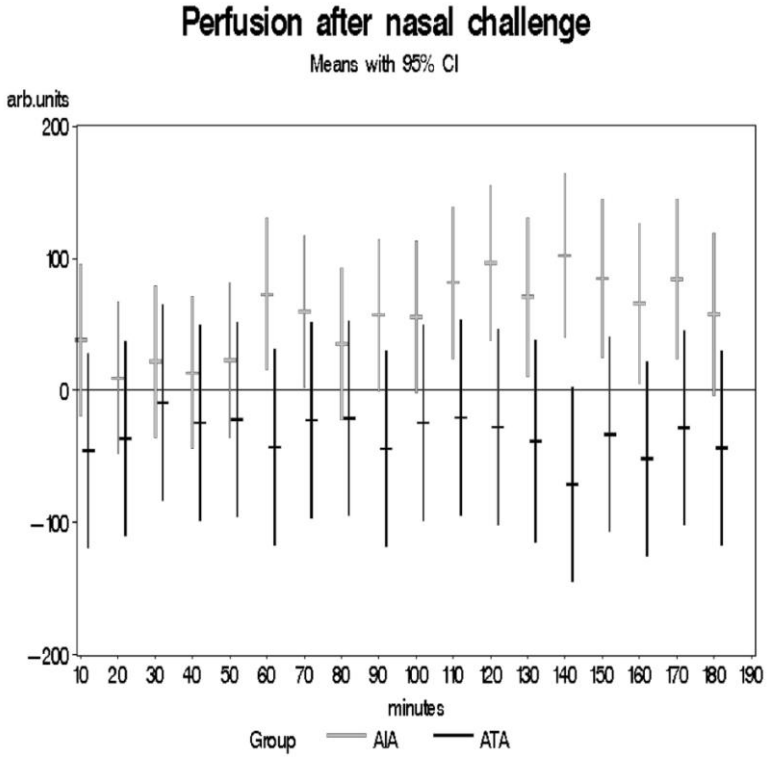


Fig. 23. The perfusion after nasal challenge with Lysine-aspirin in both groups throughout the nasal challenge test.

- The swelling was significantly increased as compared to baseline within the AIA group in the interval 50-180 minutes after spray (p= 0.05 - 0.0001). However, although the levels of swelling were higher within the AIA group throughout the entire challenge test as compared to the ATA group, there were no significant differences between the two groups 0-180 min after challenge (p= 0.24).

- PNIF was reduced as compared to the ATA group in the interval 100-130 minutes after spray (p= 0.039).

- The mean symptom scores of rhinorrhoea (Fig. 24) and patency were increased in the AIA group as compared to the ATA group in the interval
- 100-180 minutes after spray (Mann-Whitney one-sided P=0.025 and p=0.05 respectively).

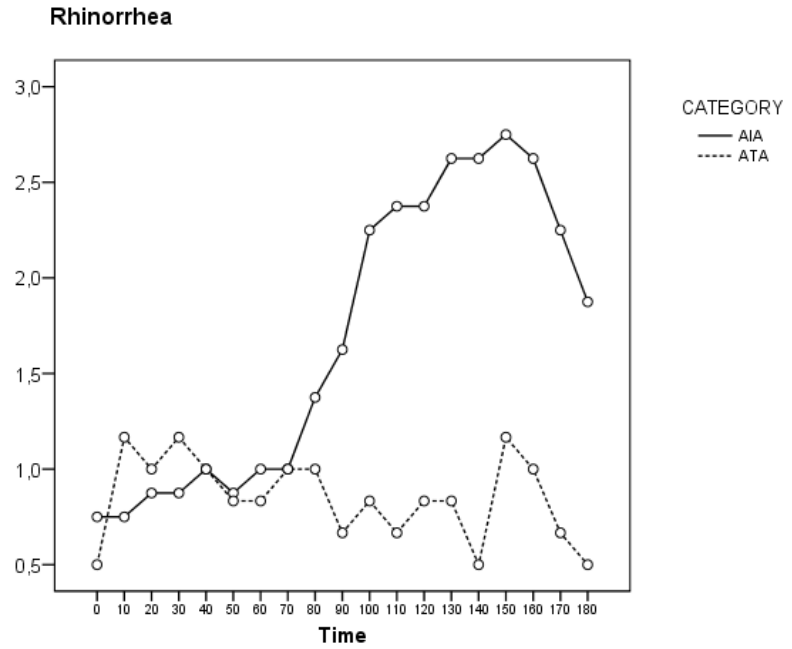


Fig. 24. The symptom scores of rhinorrhoea after Lysine-aspirin (paper V)

- The mean FEV1 was 89 – 99% of baseline throughout the challenge test in the AIA group. One of these patients developed asthma 110 minutes after nasal challenge. In the ATA group, mean FEV1 was 95–104% of baseline. However, no statistically significant differences were found between the two groups in FEV1 throughout the challenge test ($p > 0.05$).

COMMENTS

In this study, we used RSM-LDF as a method of studying the nasal mucosa after a local challenge with lysine-aspirin, and evaluated the possible differences between AIA and ATA patients in the vascular response. We also evaluated possible effects on the lower airways after a nasal challenge and vice versa, with a focus on keeping to the united airways concept.

Until 2007 when the GAL2EN recommendations were published [186], the methodology varied widely in several aspects so that it was often difficult to compare results from different studies. This study was designed prior to the publication of the GAL2EN guidelines, and therefore we did not perform a saline challenge before the aspirin challenge as recommended. According to these recommendations, if a change of greater than 20% in the recorded values occurs, then the upper airway is responsive to aspirin and a further challenge is not needed to confirm the diagnosis. Furthermore,

these guidelines recommended the challenge dose to be 16 mg lysine-aspirin, and that it should be locally instilled with an Eppendorf pipette bilaterally.

We used different doses of Lysine-aspirin, 18, 25 and 36 mg, with the purpose of finding the most efficient and safest dose. Lysine-aspirin was sprayed instead of using a pipette, with the rationale being that challenging a greater area of the nasal mucosa would activate a greater number of mast cells and thereby initiate a stronger reaction, demanding a lower concentration of lysine-aspirin. In most patients 18 mg was sufficient to elicit a nasal reaction including symptoms without generating acute asthma symptoms, and we have no evidence that 36 mg was more efficient than 18 mg. However, all ATA-patients were challenged with 36 mg in order to ensure excluding false negative results. Therefore our interpretation is that the GAL2EN recommendation of 16 mg for provocation is sufficient, and since one patient in our study developed asthma and so perhaps using a pipette for application of the lysine-aspirin liquid is safer than to spray.

The results of the study clearly show that there was a difference between groups in perfusion after spray, and that is interesting since this is new data. The nasal mucosal swelling was also increased in the AIA group, in fact at all 18 measurement times throughout the test. However, there were no statistical differences between the groups, and this might be due to false negative bronchial tests.

The main aims of this study were to evaluate whether a nasal lysine -aspirin challenge would generate a different vascular response in a group of AIA as compared to a group of ATA patients, and whether RSM-LDF might be useful in challenge tests with lysine-aspirin, equivalent or superior to previously used methods as symptom scores or PNIF. The outcome of this study supports that hypothesis, since there was a difference between groups in the microcirculation, but further controlled studies with evaluation of the reproducibility are required before a possible recommendation of RSM-LDF as a method to be used in this type of test. In addition, a controlled study will also be needed to evaluate whether the alternations in the microcirculation throughout the bronchial challenge with lysine-aspirin detected in our study are reproducible or if this was a coincidence. If these results can be reproduced, the mechanism behind it is probably a bronchio-nasal reflex [187, 188].

In our study, the nasal response to a local lysine-aspirin challenge clearly showed that for all parameters the maximum response occurred late in the test. To our knowledge,

this is the first published study evaluating the nasal and bronchial response more than two hours after a nasal challenge. The results indicate that prolonging the detection time to three hours might improve the sensitivity of the test. The reaction developed by lysine-aspirin in the AIA patients is complex and includes activation of mast cells and eosinophils, release of leukotrienes with different effects in the nasal mucosa [189]. Therefore, it seems that it takes more than two hours to elicit the maximum response of this reaction. This finding is in accordance with other complex mucosal reactions, which involves cellular activation and recruitment, such as the Type 1 allergic response with its typical early and late phases. This contrasts with a nasal challenge with histamine that generates a more rapid response due to the direct effects of this substance, as shown in Papers I-IV.

4.4 PAPER VI

RESULTS

- The daily symptoms scores in the diary of shortness of breath and cough were increased after surgery in both groups at Visits 5 and 6 (p= 0.007 - 0.029), and the daily PEFR (Fig. 13) was increased in the placebo + FPND-groups at Visits 5 and 6 (p= 0.010 and 0.031) and in the FPND group at Visit 5 only (p= 0.022) **(**Fig. 25**).**

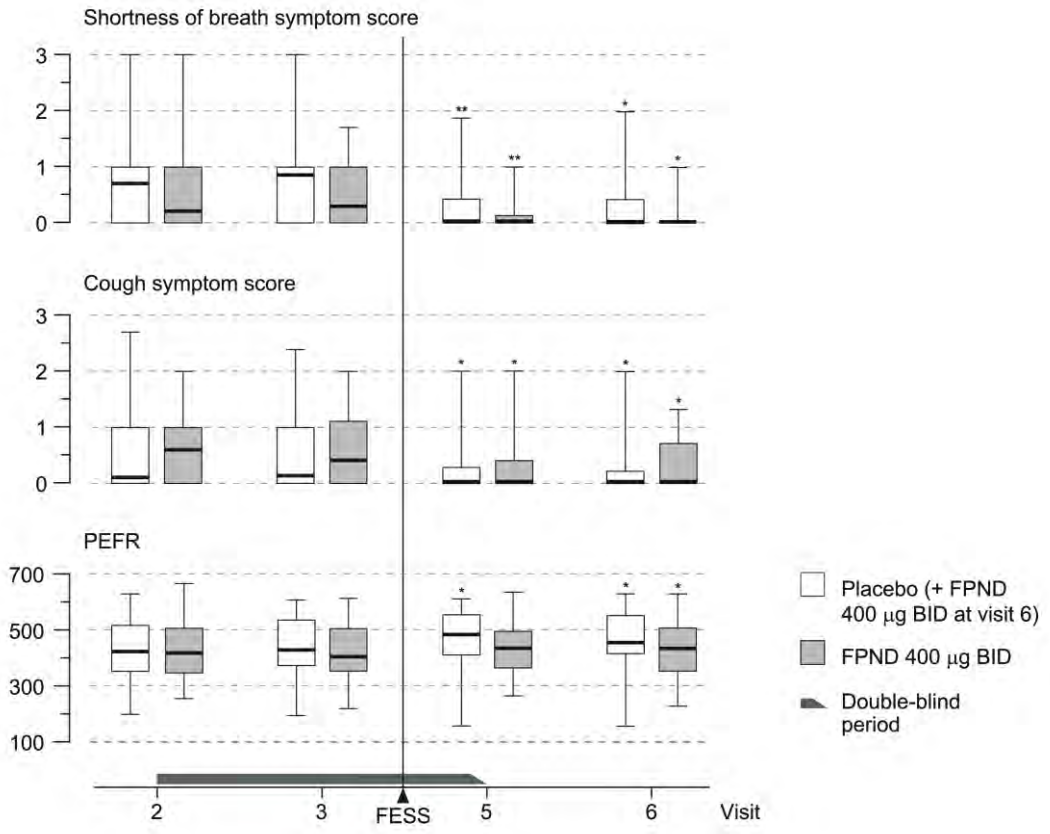
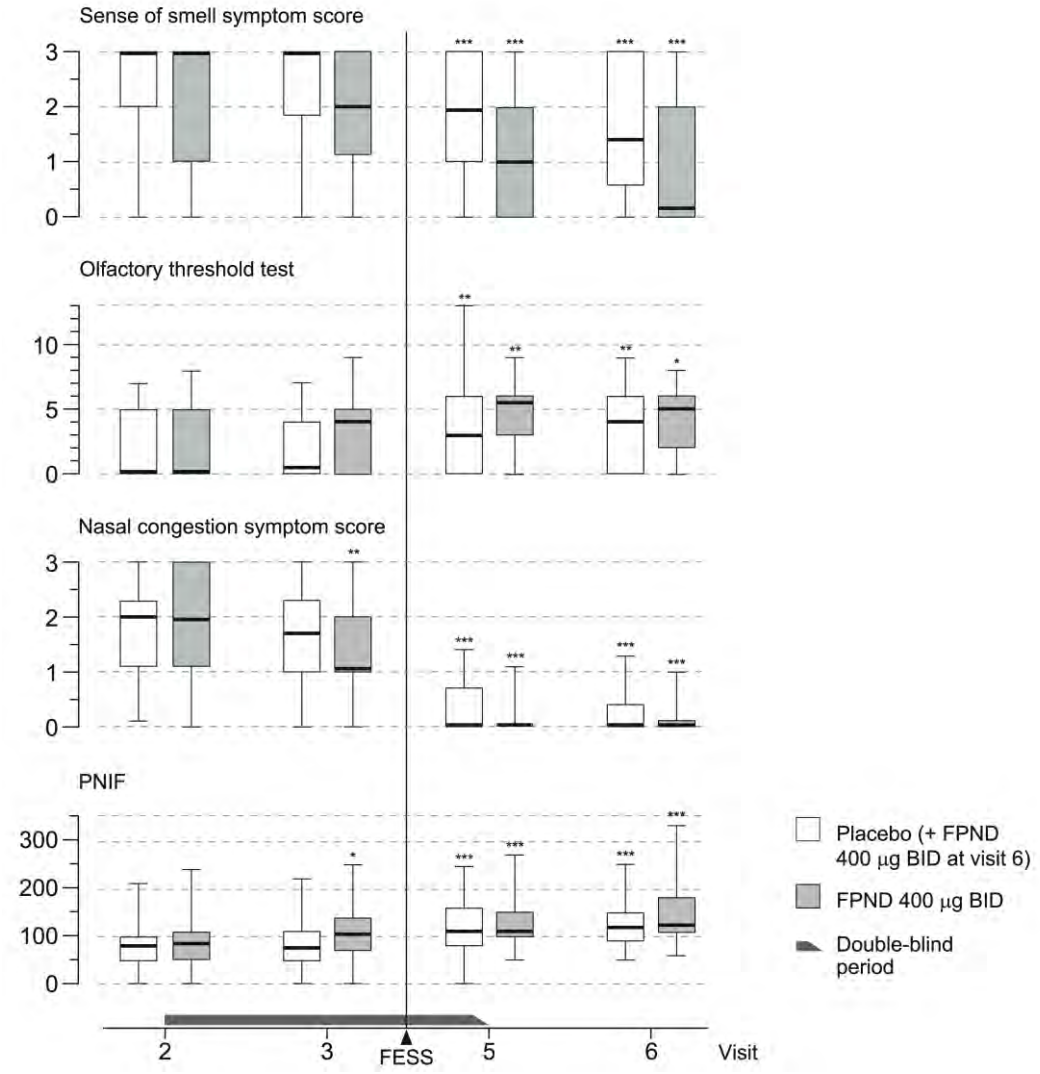


Fig. 25 Lower airways variables
*FESS reduced asthma symptoms as well as PEFR. Data is presented as median, 25% and 75% percentiles, minimum and maximum values. Statistically significant changes within groups from Visit 2 are indicated; *. P<0.05, ** P<0.01.*

- All nasal parameters, including the daily symptoms scores in the diary of sense of smell, butanol threshold test, nasal congestion, PNIF **(**Fig. 26**)**, as well as rhinorrhoea, and daily scores in the diary of nasal discharge were improved in both groups after FESS surgery (p= 0.015 – 0.001).



Text Fig. 26 Upper airways variables
*FESS reduced nasal symptoms and improved olfaction as well as PNIF. Data is presented as median, 25% and 75% percentiles, minimum and maximum values. Statistically significant changes within groups from Visit 2 are indicated; * P<0.05, ** P<0.01; *** P<0.001.*

- There were statistically significant correlations between the sense of smell vs a butanol threshold test at Visit 2 (R= -0.81, p<0.001), 3 (R= -0.85, p<0.001), 5 (R= -0.71, p<0.001) and 6 (R= 0.71, p<0.001).

COMMENTS

In this study, we continued to investigate the group of patient studied in Paper V, i.e. patients with bronchial asthma and nasal polyposis, by evaluating the benefits of FESS surgery and local nasal steroid treatment on nasal and bronchial clinical as well as subjective parameters. The objectives of the clinical management of nasal polyposis are to reduce or eliminate polyps, open the nasal airway, prevent polyp recurrence and

improve or restore patients´ quality of life [190, 191]. Clinical studies in patients with nasal polyposis have shown that FPND 400µg b.i.d. has statistically significant and clinically relevant effects on polyp size as well as on nasal congestion [192]. Therefore, medical treatment with topical corticosteroids as well as with oral steroids (OCS) - which is also used for treating asthma exacerbations [21]. According to EPO^{3}S, surgical treatment, i.e. nasal polypectomy as well as Functional Endoscopic Sinus Surgery (FESS), in nasal polyposis has not been sufficiently studied and hence has been proposed to be reserved for patients who do not satisfactorily respond to medical treatment [21]. The question whether medical and surgical treatments also have benefits on the lower airways has also been discussed, although not yet sufficiently evaluated. Predominantly positive effects have been reported in recent years from studies on the effects of surgical treatment on asthma [63, 193], but the level of evidence is low, and therefore, there is a general need for prospective randomized studies with high clinical impact upon the benefits of surgical as well as medical treatment of this patient group [21]. Except from nasal congestion and asthma, hyposmia also reduces the quality of life in this patient group. Medical treatment with steroids has been shown to have positive effects on the olfactory function, as evaluated by both subjective and clinical methods [53, 76]. Klimek et al. have reported benefits of surgery on the olfaction function, as measured by a butanol test, after including 31 patients in a prospective study, which is less than half the number of patients included in our study [194]. In that study, they used a more radical surgical method than FESS, Microscopic Endonasal Sinus Surgery (MES), including a total sphenoethmoidectomy with an enlargement of the frontal recess. Probably, OCS was not used, because "medication that might influence olfaction" was prohibited. They found a significant improvement in the olfaction function, as measured by butanol tests 4, 8 and 12, but not 24 weeks post-surgery. The olfactory function was probably better in that study, with a mean olfaction scoring of 4.19 in contrast to our study where the median value was 0. Today FESS is the gold standard among the surgery techniques, and to our knowledge no major studies have evaluated the benefits of FESS on olfaction, using clinical tests such as butanol, and therefore we also wanted to study the olfaction function in our study. OCS is a potent treatment, and combined with local steroids and surgery, OCS risks influencing the outcome. Therefore we excluded patients who throughout the study because of aggravations demanded treatment with OCS.

When designing this study, before the current EPO^{3}S guidelines were published, our hypothesis was that FESS is a potent anti-inflammatory treatment and the effects in the upper airways might also result in the reduction of lower airways inflammation. Therefore, we decided to include the whole range of patients suffering from nasal polyposis entering the clinic, from severe to mild, and with the diagnosis of bronchial asthma. Moreover, after confirming the asthma diagnosis, the pulmonologists did not alter the asthma treatment throughout the inclusion at Visit 1, if the pulmonary function was judged to be stable enough for surgery. In Sweden there is a strong tradition of treating asthmatics with inhalation steroids on a regular basis, and therefore, almost everyone was on inhalation steroids when entering the study, and the average pulmonary function was good at inclusion. Despite the fact that the patients' asthma was well controlled with inhaled corticosteroids, we noted statistically significant improvements in mean asthma symptom scores, daily PEFR (Fig. 25) with no increase in the use of β2-agonists. However, in contrast to Batra and co-workers [193], we did not find any post-surgery improvements in the spirometry as well as in the histamine challenge tests, although our patient group was larger. Our interpretation is that daily PEF scores and symptom scores registered in the diary are more relevant as an indicator of asthma severity than spirometry and histamine challenge tests performed on two single occasions in the post-surgery visits 5 and 6.

This study, which is the largest of its kind to our knowledge, provides important data on this issue, suggesting FESS should be considered early in the natural course of the disease with concomitant asthma. In addition, the study provides new data with statistically significant improvements in both objective (butanol threshold test) as well as subjective parameters of olfactory function (Fig. 3), including a statistically significant correlation between the two after endoscopic surgery. We suggest that this should be taken into consideration when evaluating FESS in patients with nasal polyposis with hyposmia.

5 GENERAL DISCUSSION

This thesis deals with diseases that affect a substantial part of the general population and may be considered as endemic modern western illnesses.

Allergic rhinitis is a global health problem affecting all countries, ethnic groups and ages, estimated to occur in over 500 million people around the world, and the prevalence is increasing in most countries (Bousquet J. et al. Allergic rhinitis and its impact on asthma [14]. The prevalence of *nasal polyposis* in Sweden is reported to be 2.7 % [195], and in studies from other countries it has been 0.5%-4%, when based upon endoscopic findings [21]. NSAID are reported to cause 21-25% of all adverse reactions [196].

Asthma is also a worldwide problem, with a global prevalence ranging from 1-18%, estimated to affect 300 million individuals with a global annual mortality of about 250 000 people (The global burden of asthma report, global initiative for asthma (GINA-report 2007: p.2). Rhinitis and asthma often coexist in the same patients [15], patients with asthma almost always have rhinitis, and rhinitis is a factor independent of allergy in the risk for asthma. [14]. Consequently, the patient groups studied in this thesis are of considerable size throughout the world, with both social and economic consequences of the airway disease, such as major illness and disability with reduced ability to work and to study as well as a decrease in the quality of life.

In Papers I and II, we found that the histamine-induced nasal mucosal swelling as measured by RSM increased in patients with allergic rhinitis regardless of whether they were exposed to allergen or not. This data is in line with those of a previous study where the nasal mucosal swelling was also increased in ten allergy patients out of pollen season as compared to a group of healthy subjects [174]. This data is from small patient groups but is in agreement with the hypothesis that the increased swelling out of pollen season could be due to a minimal persistent inflammation [173, 197]. In contrast to these findings, healthy subjects after exposure to swine dust showed an increased histamine sensitivity as measured by RSM,

Accumulating evidence suggests that even intermittent allergic rhinitis is a chronic inflammatory disease instead of a disease of acute symptoms, and even when symptoms are absent, a minimal level of persistent inflammation may persist [198, 199]. In patients sensitized to house dust mites but without allergic symptoms,

increased levels of inflammatory cells were found, in particular neutrophils but also eosinophils, and a mild ICAM-1/CD 54 expression in scrapings from the nasal mucosa and conjunctiva, in contrast to relatives and healthy subjects, who showed no such expression and only few neutrophils but no eosinophils [198]. The C54 (ICAM-1) molecule is also the major receptor for human rhinoviruses [200], which may partially explain the relationship among allergy, viral infections, and asthma [173]. In patients with seasonal allergy, a significant inflammatory reaction, with increased levels of ICAM-1, eosinophils and neutrophils, was also evident during the days with a low pollen count and low or absent symptoms [201]. Therefore, a minimal persistent inflammation has now become an important hypothesis, and it has also been suggested that the consequences would be to evaluate a new therapy strategy in order to prevent unexpected exacerbations and achieve effective control of airway inflammation [197, 199].

Acute inflammation usually resolves with normal repair processes, but with chronic inflammation the repair process is disturbed, leading to remodelling [202]. This process involves a thickening of the airway walls due to subepithelial fibrosis, myocyte hyperplasia and hypertrophy, myofibroblast hyperplasia, mucus gland and goblet hyperplasia [203], infiltration with eosinophils and T-cells in the mucosa and submucosa, leading to oedema [96]. In allergic rhinitis, remodelling is still poorly understood, and even though inflammation is similar in allergic rhinitis and asthma, the pathological extent of nasal remodelling may be different from those of the bronchi [14]. In contrast to the bronchi, despite hypervascularity, epithelial damage is only minimal in the nasal mucosa of patients with allergic rhinitis, and epithelial shedding is more pronounced in the bronchi than in the nose of the same patients suffering from asthma and rhinitis [14].

The model we used for induction of airways inflammation in healthy subjects is quite simple, efficient, relatively short-lived and cheap, and in contrast to nasal pollen provocation (in the group of pollen allergy patients, Papers I and II), swine dust exposure induces a concomitant inflammation in both upper and lower airways. Therefore it has clear-cut advantages as compared to an alternative method, such as inducing the common cold, and might be a tool for comparing the nasal and pulmonary function, including the hyperresponsiveness, in healthy subjects with that of pollen allergy patients out of and throughout pollen season. However, when comparing the

outcome of these studies it is important (Papers 1, II vs. Papers III, IV) to bear in mind that there is a different type of inflammation after exposure to swine dust in healthy volunteers compared to that after pollen exposure in allergy patients. The main difference is that swine dust exposure recruits neutrophils via an innate immune response with Toll-receptor activation and a cognate response with the secretion of TH1 cytokines, whereas IgE-mediated inflammation recruits eosinophils through TH2 mechanisms [204]. However, there are also some connections between these, as some studies have shown a relation between IgE-mediated and neutrophil inflammation [204] Thus, LPS-induced neutrophil inflammation can both promote and counteract the development of an IgE-mediated inflammation, depending on the dose, the subject´s age and the manifestation of the allergy [25, 204, 205]. Moreover, increased levels of eosinophils have been detected in NAL following nasal endotoxin challenge, and in BAL following swine dust exposure [25, 205].

The previous investigations performed in connection with swine dust exposure have mainly focused on the lower airways, and therefore our work contributes by studying the nasal response, including measurements of nasal mucosal swelling and microcirculation and symptom scores, which are methods never used for studying the effects of swine dust exposure, but also by keeping to the united airways perspective. Therefore, we also used methods such as NAL, spirometry and the bronchial histamine challenge test, which also confirmed the reproducibility of previous findings.

In Papers III and IV we also found that, clearly NAL had significant effects on the evaluation of nasal mucosal swelling, microcirculation as well as symptoms, since even a small volume of saline (5 + 5 ml) after exposure to swine dust had significant effects on the outcome of the histamine sensitivity. This study design was constructed mainly for scientific purposes, in order to evaluate whether these investigations would interfere, and the outcome is that they do. These investigations should obviously be kept separated in time.

These strong effects of saline may be due to the evacuation of irritants as well as inflammatory agents, and shed light on nasal irrigation as a method for anti-inflammatory treatment. Nasal irrigation is a simple and cheap method, used to treat sinus and nasal conditions for many years [185], routinely recommended among otorhinolaryngologists [206]. The method is safe and well tolerated, even in children [207], with few adverse reactions [207, 208]. Different studies of nasal irrigations report the benefits in managing sinonasal complaints in a variety of conditions such as

acute and chronic rhinosinusitis, allergic and non-allergic rhinitis, septal perforations and postnasal drip [184, 185]. Therefore in several countries the method has been recommended as an adjunct therapy in guidelines for all causes of rhinosinusitis, including post-operative cleaning of the nasal cavity in treatment guidelines [185]. Nasal irrigation promotes ciliary function and reduces oedema, which would improve drainage through the sinus ostia [209]. In fact, the method may be underutilized, and it has been proposed that nasal irrigations should no longer be considered as an adjunctive therapy in managing sinonasal conditions [184]. However, no standard uniform recommendations exist for the use of nasal irrigations and the methodology, such as the administering device and the tonicity (isotonic or hypertonic saline), varies between studies [185]. Consequently, the level of evidence is often low, and therefore the results of this study might make a contribution to the previous findings.

[21] Diagnosing aspirin sensitivity is important, because an adverse reaction may be fatal for the AIA patient, and that diagnosis excludes the use of the entire group of NSAID. Today, there is no in vitro test recommended for this purpose [21]. Therefore, a precise history in combination with a challenge test is required to achieve the safest diagnosis. In Paper V we used two of the three different challenge methods, currently recommended as a diagnostic tool for aspirin intolerance [21, 186], The nasal challenge test has a slightly lower sensitivity (73%) than the other two other tests (77%), and is therefore recommended especially for patients with predominantly nasal symptoms and those in whom the oral or bronchial challenge test is contraindicated because of asthma severity [21]. The AIA patient group often has more severe asthma than other asthmatics, and this is a particular reason for increasing the sensitivity of this method. We found that the magnitude of the nasal response was strongest more than two hours after challenge (Paper V), which is interesting because other publications on this issue have reported a maximum detection time of at most two hours after challenge [210-214]. An extended time measurement might improve the sensitivity of the test and thereby contribute to increasing the usage of the nasal challenge.

The physiology of the nose is complex, and today there is no single method for detecting the nasal response in nasal challenge tests comparable to spirometry and the calculation of PD20 in a bronchial challenge test. Instead, it appears that combining different methods for detecting the nasal response to a lysine-aspirin challenge is

necessary for the best outcome. Consequently, we found it interesting that there were differences between groups in the microcirculation after nasal challenge, and if this data is reproducible RSM-LDF might be added to the recommended methods.

The recommended treatment strategy for nasal polyposis is anti-inflammatory, with a recommendation for topical and oral corticosteroids, and surgical treatment is proposed to be reserved for patients who do not satisfactorily respond to medical treatment [21]. This is because the benefits of surgical treatment have not yet been sufficiently studied, and therefore our study is intended to contribute in this aspect. The current basal anti-inflammatory treatment in this patient group is daily topical treatment with steroids, and therefore we found it relevant to evaluate the surgical treatment (FESS) with or without concomitant treatment with local steroid or placebo treatment by randomizing the groups to either FPND or placebo, but include surgery in both groups. For some patients, four weeks of wash-out from nasal steroids before surgery (between Visits 1 and 2) caused heavy local symptoms forcing them to withdraw participation of the study. We did not also include wash-out from asthma medication in the study design, because this would probably have further increased the withdrawal from the study group. The difficulties in completing a wash-out period from treatment is also an illustration of the magnitude of the airway symptoms in this patient group.

Rhinitis, and in particular nasal polyposis, is associated with an impaired sense of smell, and this may be due to mucosal obstruction of the olfactory niche and/or degenerative alterations in the olfactory mucosa due to the disease [21]. In Paper VI we found that FESS improved the olfactory function, as evaluated by both subjective scoring and by a butanol threshold test. This is interesting because no major study has been performed until now that demonstrates objective effects on the sense of smell after FESS in nasal polyposis [194], and repeated sinus surgery is meant to be a risk factor for hyposmia [21, 215].

Subjective scoring of olfaction is a commonly used assessment method, and in validating clinical settings, subjective scores have been found to significantly correlate to objective measurements of the olfactory function [54, 57] , and in Paper VI we also found a significant correlation between subjective scoring and the butanol threshold test. Earlier studies have shown that topical corticosteroid treatment, FPND, as well as Mometasone Furoate Nasal Spray, in nasal polyposis had statistically significant and clinically relevant effects on the subjective sense of smell [75, 76, 192]. OCS has

significant effects on both the subjective as well as the clinical olfaction function [53]but is associated with adverse reactions with negative systemic effects on the hypothalamic-pituitary-adrenal (HPA) axis and osteoporosis [86]. Our results with positive effects on both clinical and subjective olfaction might to some extent contribute to altering the indication for FESS in patients with nasal polyposis and hyposmia.

In Paper II we found an increased bronchial hyperresponsiveness in a group of pollen allergy patients, and a correlation between upper (the number of sneezes) and lower (PC20 PEF) airways histamine sensitivity. In Paper V we also found microcirculatory changes in the nasal mucosa during the time span from 60 minutes before to 60 minutes after the AIA patients developed asthma, suggesting the presence of a bronchio-nasal reflex. In Paper VI both symptom scores of asthma and the daily PEFR improved after FESS. These findings fit in with the united airways concept, which postulates that connections also exist in other ways than only the anatomic proximity.

Based on knowledge from epidemiologic and clinical studies, it is logical to assume that airway allergy is not a disease confined to a specific target organ, but rather a disorder of the whole respiratory tract, a systemic disease with a broad spectrum of clinical manifestations [18]. This perspective also concerns the other patient groups in this thesis, asthmatics with concomitant nasal polyposis with or without NSAID intolerance, because nasal polyposis is associated with bronchial asthma. 7 % of asthma patients have nasal polyposis, and this connection is stronger (10-15%) with a late onset of asthma [21, 132]. The therapeutic consequences of the close connections between allergic rhinitis and asthma have been concluded in the Allergic Rhinitis and its Impact on Asthma (ARIA) initiative, which recommends that the presence of asthma must be considered in all patients with rhinitis, and that in planning treatment, both should be considered together [14].

The mechanisms behind this close connection between upper and lower airways are not fully understood, but there are some theories that try to explain the mechanisms behind: Nasal provocation with petrolatum packing [216] and cold air [217] has been reported to initiate bronchoconstriction. Nasal provocation with histamine has been reported to both initiate an immediate bronchconstriction suggesting a *naso-brochial reflex* [218] as well as no bronchial reaction [16]. Nasal allergen provocation altered the lower airways caliber and the nonspecific bronchial responsiveness to methacholine [150],

while in other studies no effects on the lower airways function were found after a nasal allergen provocation. *A systemic distribution* of nasal to lower airway inflammation, migration of eosinophils [153] *Postnasal drip* with aspiration of inflammatory secretions from the upper airways into the lower airways. In an experiment with induced granulocytic rhinosinusitis in rabbits, the bronchial histamine responsiveness increased although the baseline pulmonary function was unaltered, and these changes in reactivity were blocked by strategies that prevented the exudate from draining beyond the larynx [219]. It can be speculated that postnasal drainage of inflammatory cells, in particular during sleep, may affect lower airway responsiveness [16].
Nasal ventilation minimizes airway cooling in both normal and asthmatic individual through more efficient conditioning of inspired air, and it is through this mechanism of a more effective *humidification and warming of inspired air, as well as a filter effect* that this form of respiration protects against exercise-induced bronchospasm [220]. We do not know whether one of these mechanisms is more important than the others, and it is likely that more than one of the mechanisms described above contributes to linking the nose to the bronchi, i.e., alterations in lung function in patients with rhinitis.

It was not long ago that there was a debate among physicians whether or not rhinitis had a significant impact on asthma. Obviously, knowledge about this issue has increased over the last few decades, and therefore it would be reasonable to expect that it also would be put into clinical practice. A consequence would be that the ENT specialist should investigate the history of the lower airways as a routine in the patients with chronic and acute rhinosinusitis, and should also focus on examining the pulmonary status with a stethoscope and a spirometer. Conversely, the pulmonologist ought to investigate the asthma patient for the possible co-existence of rhinosinusitis, and be familiar with examining the nose with a nasal specula including decongesting the nasal mucosa, and why not even a nasoscope. However, there are borders to cross, perhaps between both the different specialities and the surgical and internal medicine traditions. It is necessary to integrate the consequences of the present knowledge about this topic in everyday clinical practice if we are to treat these patients in the best way.

6 CONCLUSIONS

1. Nasal histamine reactivity in pollen allergic patients as measured by nasal mucosal swelling was not influenced by pollen exposure during season or challenge out of season, and neither was bronchial histamine reactivity increased under pollen season. This indicates the presence of a persistent inflammation in patients with seasonal allergy.
2. Nasal and bronchial histamine reactivity in healthy subjects, as measured by both nasal mucosal swelling and microcirculation (CMBC), was increased after exposure to swine dust.
3. Nasal lavage before a histamine challenge affected the outcome of nasal mucosal swelling, the microcirculation as well as nasal patency, which implies this has to be kept in mind when studies are designed.
4. Measurement of CMBC confirms RSM-LDF as a non-invasive method for the detection of interstitial oedema.
5. RSM-LDF may be useful as an alternative method for detecting aspirin sensitivity in nasal challenge tests.
6. A three hour observation time after a nasal lysine-aspirin challenge may be recommended, as the maximal response of both subjective and clinical parameters occurred late in the test.
7. FESS may be considered early in the natural course of the disease with concomitant asthma.
8. FESS may also be considered a second-line treatment in patients with a reduced sense of smell and nasal polyposis.

7. POPULÄRVETENSKAPLIG SAMMANFATTNING

Rhinit och astma orsakas av inflammation i luftvägarna och är folksjukdomar som orsakar lidande och stora ekonomiska kostnader över hela världen. Det här avhandlingsarbetet utgörs av resultaten från sex olika kliniska publikationer där patienter och friska försökspersoner med olika typer av luftvägsinflammation studerats: pollenallergi, neutrofil luftvägsinflammation orsakat av svindammsexponering efter arbete i en anläggning för grisuppfödning, samt astma och näspolypos med eller utan överkänslighet mot aspirin och andra anti-inflammatoriska värktabletter. Syftet med avhandlingen var att studera olika aspekter av luftvägsinflammation i övre och nedre luftvägarna med speciellt fokus på eventuella samband mellan övre och nedre luftvägsinflammation. Resultaten är presenterade i sex separata publikationer.

Hyperreaktivitet i luftvägarna innebär en ökad känslighet för olika fysikaliska, kemiska och fysiologiska stimuli, t.ex. parfymdoft, tobaksrök, kall luft och ansträngning. I delarbete I-IV använde vi histamin i både näsa och bronker för att påvisa hyperreaktivitet. Vi mätte svullnadsförändringar i nässlemhinnan och förändringar i lungfunktionen med rhinostereometri respektive spirometri.

I delarbete I och II fann vi en ökad histaminkänslighet i näsan året runt hos pollenallergiker, jämfört med en grupp friska försökspersoner. Vi fann också ett samband mellan histaminkänslighet i näsa och i bronker. En hypotes som skulle kunna förklara den ökade känsligheten för histamin året runt är att inflammationen i slemhinnan finns kvar även utanför pollensäsong s.k.”minimal persistent inflammation”, ett fenomen som diskuteras i samband med allergisk inflammation.

I delarbete III och IV utsattes friska försökspersoner för svindamm, vilket orsakar en kraftig luftvägsinflammation. Vi fann att histaminkänsligheten ökade i både bronker och nässlemhinna (svullnad och mikrocirkulation) efter att försökspersonerna exponerats för svindamm. I de här två arbetena använde vi nässköljning som en metod för att samla in inflammatoriska celler och markörer. Vi fann att nässköljningen minskade hyperreaktiviteten i näsan, vilket talar för att klinisk användning av nässköljning har en antiinflammatorisk effekt.

I delarbete V användes vattenlösligt aspirin, lysin-ASA, för att påvisa känslighet för aspirin och andra antiinflammatoriska värkmediciner hos en grupp astmatiker med näspolypos. Vi använde en i sammanhanget ny metodik, rhinostereometri med laser Doppler mätningar. Vi fann att gruppen som var känsliga för bronkiell provokation

med lysin-ASA fick en ökad reaktion vid nasal provokation, jämfört med en kontrollgrupp. Huvudreaktionen i näsan kom två timmar eller senare vilket tidigare inte har varit känt. Vi fann också att provokation med lysin-ASA i lungorna gav en mätbar reaktion i näsans mikrocirkulation.

I delarbete VI studerade vi effekten av näs-bihålekirurgi (FESS) och lokal behandling med kortisonnäsdroppar på en grupp patienter med näspolypos och astma. Vi fann att näs-bihålekirurgi förbättrade patienterna nässymtom, inklusive luktsinnet, vilket tidigare inte visats. Patienternas lungsymtom och lungfunktion förbättrades också. Den här effekten av kirurgi har tidigare diskuterats. Hittills har FESS rekommenderats som sista behandlingsalternativ då medicinsk behandling inte fungerat. Våra resultat framhäver kirurgisk behandling som en effektiv behandlingsmetod vid näspolypos med effekt på såväl övre som nedre luftvägar. Man kan diskutera om kirurgi bör användas tidigare i sjukdomsförloppet än idag för behandling av patienter med näspolypos med astma/luktnedsättning. I vår studie hade behandling med kortisonnäsdroppar ingen effekt på patienternas astma.

Fynden att näs-bihålekirurgi förbättrade patienternas astmasymtom och lungfunktion, att vi fann ett samband mellan histaminkänslighet i näsa och bronker hos pollenallergiker och att vi kunde påvisa mätbara förändringa i näsan vid bronkprovokation med lysin-ASA är exempel på att näsa och bronker är förenade i ett gemensamt funktionellt system, vilket ofta benämns ”the united airways”.

8 ACKNOWLEDGEMENTS

Acknowledgements

There are many people who have contributed to the work presented in this thesis, and to whom I am grateful. Besides the patients and volunteers participating in the studies, I would particularly like to mention the following people:

My supervisors:

Associate Professor Pär Stjärne

Tutor and head of the ENT-department, for his genuine, infectious and friendly dedication, and sharing his broad knowledge in this research field, with a natural, open-minded, generous and supportive way of being. You are a real wonderful guy, Pär!

Professor Jan-Erik Juto

For his constant enthusiasm and passion, and for opening up my eyes to the joy and stimulus connected with rhinological research. Also for his cleverness both in medical as well as technological issues, this combination resulted in the rhinostereometer apparatus, used in Papers I-V.

Associate Professor Barbro Dahlén

For sharing her impressive knowledge about the asthma disease, bronchial hyperresponsiveness, airway inflammation, challenge tests as well as aspirin intolerance.

My co-workers:

Dr. Karl-Gustav Kölbeck

My much-appreciated friend and co-worker on all six papers, with our cooperation building a bridge between the upper and lower airways, which is the fundament for the united airways concept of this thesis.

Dr. Petter Olsson

For bringing structure, knowledge, friendship, energy, supportiveness and not to forget, a good helping of humour and laughs when struggling with the last study. Hopefully, this job will be a start of future research cooperation.

Dr. Hans Grudemo
For constructing the technical innovation of the RSM-LDF apparatus in cooperation with Jan-Erik Juto and performing the necessary first jobs, and for sharing this knowledge and thereby paving the way for the results presented in Papers IV and V.

Dr. Björn Mossberg
For introducing me into the difficult art of writing a scientific paper in proper English.

Dr Maria Skedinger
One of the five Pulmonologist colleagues participating in this thesis for contributing with her valuable knowledge and careful work in the last study.

Professor Kjell Larsson and Dr Lena Palmberg
For sharing their model with exposure to swine dust, by weighing pigs in a confinement building, an essential piece of cooperation for the origin of Study 2 (Papers III and IV).

Dr Sune Forsberg and Hans Gyllenhammar
For contributing with NO measurements in Paper III.

Martin Åhlenius
My favourite statistician, with the talent of making the statistical way of thinking quite understandable, in a friendly manner, and always delivering high quality punctually.

Ann-Sofie Lantz, Agneta Gülich and Marianne Eduards
Nurses at the FoU unit at the Department of Respiratory Medicine and Allergy at Karolinska Hospital, Huddinge, for their generous help in all kind of practical as well as theoretical issues concerning aspirin and histamine bronchial challenge tests, with the results being published in Papers V and VI.

Vania Sandberg and Christa Elgerius
Respectively nurse and biomedical analyst in the ENT outpatient ward at Karolinska Hospital, Huddinge, for in practice bringing the last study into port with a lot of energy, as well as their warm and professional approach to the patients. With the ability to

motivate the patients to complete the study, and make them feel comfortable and particularly well treated despite their often severe airways symptoms.

Professors:

Professor Lars Olaf Cardell

For bringing remarkable energy and brilliance, thereby increasing enthusiasm for research at the clinic after only a short period of duty as a professor at Karolinska Institutet.

Professor Pontus Stierna

For his professional support and friendship, always with a humorous twinkle in his eye

My colleagues, medical as well as other profession, and friends:

Colleagues in the rhinology group:

Doctors Michael Ohm, Karin Toll, Peter Graf, Hans Hallén, Jan Falk, Urban Knutsson and Gert Henriksson

,For stimulating discussions and cheerful cooperation.

The collegues and friends at Nacka Närsjukhus:

Including Per Granstrand, Maya Larsson Boman, Charlotte Falkenberg, and the managerial group of Nacka Närsjukhus Proxima

For patience with my research job as well as an flexible attitude to finding solutions for combining the clinical and research job.

Dr Ann Abrahamsson

For acting as my substitute at the ENT-ward in Nacka Närsjukhus, thereby helping me to complete this thesis.

Agneta Wittlock

For helping with her excellent secretarial assistance.

Tony Carlyle

For checking every word in the text and correcting the English language whenever requested, including during weekends and vacation.

Ludvig Stjärne
For generous contribution with illustation and Figures in paper V and VI.

Malin Edlund
Photographer, for professional and generous help with the illustrations, including the cover of the book.

Björn Ehnhage
My brother, for participating with his allergic nose, and in particular for rescuing me several times when trapped in the pitfalls of Excel.

My family:
Kerstin, Malin, Sara, Anna and Linn, as well as my parents, Gunnar and Unni and sister Nina
For all manner of support, such as letting me use their noses so I could practise measurement techniques before meeting the patients, for making professional photographs as well as model jobs for the book, for being indulgent towards my absentmindedness, and for a lot of other help, not to forget different arrangements in the final phase of this effort.
This is despite the fact that I may not always have been able to answer the natural questions: ”Why are you doing this?”, and ”When will you be ready?”

This thesis was supported by grants from The Swedish Asthma and Allergy Foundation (74X-15067), The Swedish Heart & Lung Foundation, The Swedish Council for Work Life Research (94-1383), The Swedish Association of Otorhinolaryngology, Head and Neck Surgery, the Acta Otolaryngologica Foundation, Praktikertjänst AB and GlaxoSmithKline.

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