REVIEW

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EP^{3}OS 2007: European position paper on rhinosinusitis and nasal polyps 2007. A summary for otorhinolaryngologists*

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INTRODUCTION

Rhinosinusitis is a significant health problem, which seems to mirror the increasing frequency of allergic rhinitis and which results in a large financial burden on society [1-3]. Data on (chronic) rhinosinusitis are limited and the disease entity is badly defined. Therefore, the available data are difficult to interpret and extrapolate.

The last decade has seen the development of a number of guidelines, consensus documents and position papers on the epidemiology, diagnosis and treatment of rhinosinusitis and nasal polyposis [4-7]. In 2005 the first European Position Paper on Rhinosinusitis and Nasal Polyps (EP^{3}OS) was published [8, 9]. This first evidence based position paper was initiated by the European Academy of Allergology and Clinical Immunology (EAACI) to consider what was known about rhinosinusitis and nasal polyps, to offer evidence-based recommendations on diagnosis and treatment, and to consider how we can make progress with research in this area. The paper has been approved by the European Rhinologic Society.
Since the preparation of the first EP^{3}OS document an increasing amount of evidence on the pathophysiology, diagnosis and treatment has been published (Figure 1).

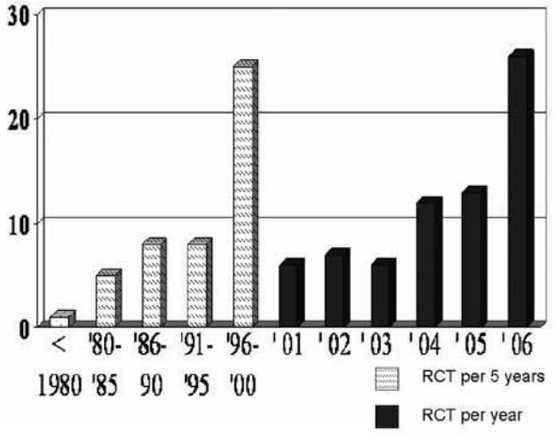

Figure 1. Randomized controlled trials in chronic rhinosinusitis with or without nasal polyps. The number of trials in the last 5-6 years equals the number ever published before.

In the 2007 revision [10] new data have led to a considerable increase in amount of available evidence and therefore to considerable changes in the schemes for diagnosis and treatment. Moreover, the whole document has been made more consistent, some chapters, like the one on surgery, are significantly extended and others are added. Last but not least contributions from many other parts of the world have attributed to our knowledge and understanding.
This summary indicates the main differences between the first EP^{3}OS document and the EP^{3}OS 2007 with emphasis on definition, diagnosis and treatment of CRS by otorhinolaryngologists.

RHINOSINUSITIS DEFINITION

Rhinitis and sinusitis usually coexist and are concurrent in most individuals; thus, the correct terminology is now 'rhinosinusitis'. The diagnosis of rhinosinusitis is made by a wide variety of practitioners, including allergologists, otolaryngologists, pulmonologists, primary care physicians and many others. Therefore, an accurate, efficient, and accessible definition of rhinosinusitis is required. The paper again gives different definitions for epidemiology, first line and second line treatment and for research. Here we summarize the relevant changes for otorhinolaryngologists.

Clinical definition of rhinosinusitis

Rhinosinusitis (including nasal polyps) is defined as inflammation of the nose and the paranasal sinuses characterised by two or more symptoms, **one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip)**, ± facial pain/pressure, ± reduction or loss of smell; and either endoscopic signs of polyps and/or mucopurulent discharge primarily from middle meatus and/or; oedema/mucosal obstruction primarily in middle meatus, and/or CT changes showing mucosal changes within the ostiomeatal complex and/or sinuses.

The definition has been sharpened by indicating that at least nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip) should be a symptom of rhinosinusitis.

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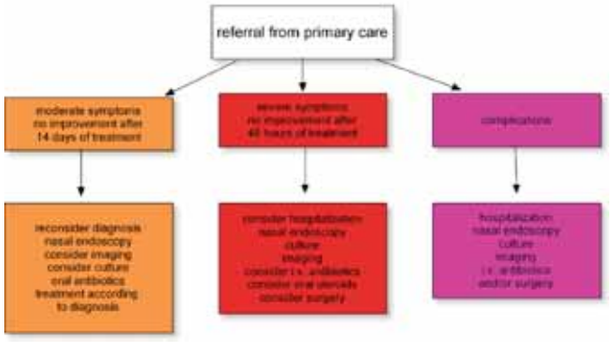

Figure 2. Management scheme for ENT specialists for adults with acute rhinosinusitis.

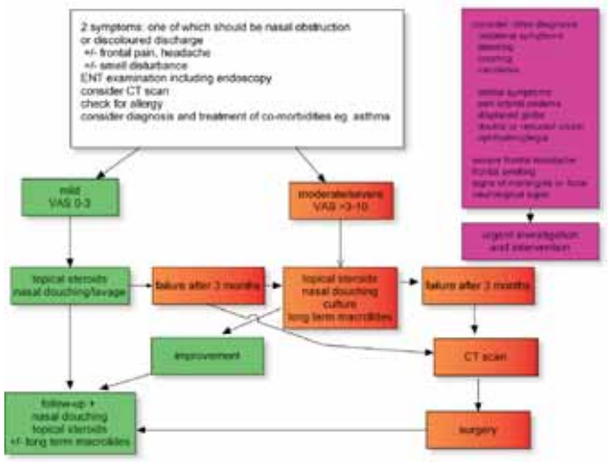

Figure 3. Management scheme for adults with CRS without NP for Otorhinolaryngologists.

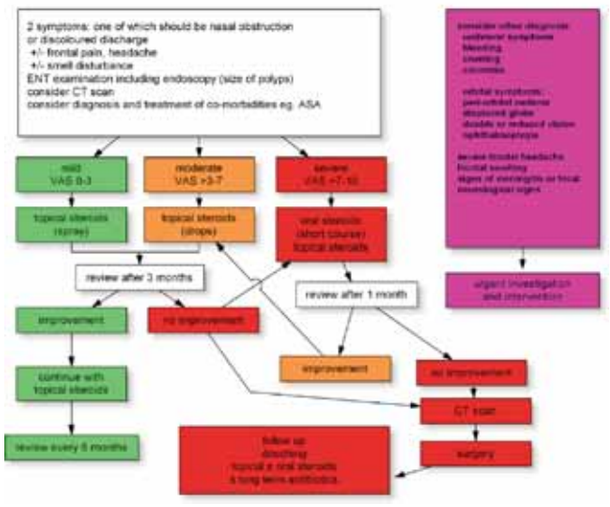

Figure 4. Management scheme for adults with CRS with NP for Otorhinolaryngologists.

Table 1. Treatment evidence and recommendations for adults with acute rhinosinusitis.

Therapy	Level	grade of recommendation	Relevance
oral antibiotic [32]	Ia	A	yes: after 5 days, or in severe cases
topical corticosteroid [33]	Ib	A	yes
topical steroid on top ofand oral antibiotic [34, 35] combined	Ib	A	yes
oral corticosteroid [36]	Ib	A	yes reduces pain in severe disease
oral antihistamine [37]	Ib	B	yes, only in allergic patients
nasal douche [38]	Ib (-)#	D	no
decongestant [39, 40]	Ib (-)#	D	yes, as symptomatic relief
mucolytics [41]	none	D	no
phytotherapy [42]	Ib	D	no

: Ib(-) study with a negative outcome

Table 2. Treatment evidence and recommendations for adults with chronic rhinosinusitis without nasal polyps. *

Therapy	Level	grade of recommendation	relevance
oral antibiotic therapy short term < 2 weeks [43]	Ib (-)	C	no
oral antibiotic therapy long term > 12 weeks [44, 45]	Ib	A	yes
antibiotics – topical [46]	III	D	no
steroid – topical [47]	Ib	A	yes
steroid – oral	no data	D	no
nasal saline douche [48, 49]	Ib	A	yes
decongestantdecongestant oral / topical	no data	D	no
mucolytics [50]	III	C	no
antimycotics – systemic [51]	Ib (-)#	D	no
antimycotics – topical [52, 53]	Ib (-)#	D	no
oral antihistamine in allergic patients	no data	D	no
protonproton pump inhibitors	no data	D	no
bacterial lysates [41]	Ib	A	no
immunomodulators [54]	Ib (-)#	D	no
phytotherapy phytotherapy	Ib (-)#	D	no
anti-leukotrienes	III	C	no

* Some of these studies also included patients with CRS with nasal polyps

* Acute exacerbations of CRS should be treated like acute rhinosinusitis

: Ib(-) study with a negative outcome

Severity of the disease

The disease can be divided into mild (VAS 0-3), moderate (VAS > 3-7) and severe (VAS > 7-10) based on total severity visual analogue scale (VAS) score (as indicated by the patient). A VAS > 5 affects patient QOL [11].

Duration of the disease

Acute RS means symptoms or signs of RS for less then 12 weeks with complete resolution of symptoms. Chronic RS means symptoms of signs of RS for more than 12 weeks without complete resolution of symptoms. Chronic rhinosinusitis may also be subject to exacerbations.

EVIDENCE BASED SCHEMES FOR DIAGNOSTIC AND TREATMENT

The tables (Table 1-3) and schemes (Figure 2-4) for diagnosis and treatment are the result of a critical evaluation of the available evidence. The tables give the level of evidence for studies with a positive outcome and well powered studies with negative outcome. Ib (-) in this tables means a well-designed (Ib) study with a negative outcome. The grade of recommendation for the available therapy is given. Under relevance, the group of authors indicate whether they think this treatment to be of relevance in the indicated disease. The references given in the summary are examples of studies of the highest level. If many studies are available only a few recent studies are referred. The full list of references can be found in the full document [10].

EVIDENCE BASED SURGERY FOR RHINOSINUSITIS

In EP³OS 2007 systematic reviews on sinus surgery efficacy in CRS are presented, followed by a description of comparative trials of sinus surgery with medical treatment. The role of various surgical modalities is briefly reviewed, and reports on the effects of concomitant diseases on sinus surgery outcomes are detailed.

It is difficult to generalise about sinus surgery studies because surgery is indicated in the full document selected patients who are not sufficiently responsive to medical treatment. Moreover, only a few publications on sinus surgery qualify for evidence based evaluation [12] and frequently studies included in systematic reviews are assigned low evidence levels [13-15]. This is in part due to specific problems in conducting surgical trials. In general, surgery is difficult to estimate or standardize, particularly in multi-centre trials, and the type of treatment is difficult to conceal (blinding). Randomization may pose ethical problems unless narrow inclusion criteria are set [16].

In addition, a variety of confounders make it difficult to obtain homogenous patient groups with comparable therapeutic procedures for unbiased evaluation of sinus surgery outcomes. Possible relevant surgical factors include whether an external or endonasal approach is chosen, whether a functional or conventional surgical procedure is selected, if the extent of the surgical intervention is limited, extended or radical, and what kind of instruments are employed. Patient-dependent factors include age, extent and duration of disease, previous surgery, presence of polyps, concomitant diseases such as ASA-intolerance, asthma, or cystic fibrosis, and particular aetiologies including dental, autoimmune, immune, and fungal disease [17-20]. Moreover, mode and duration of pre- and post-operative drug therapy may alter the outcome.

Taken these limitations into account, however, a significant amount of data is available on the efficacy of surgery in CRS with or without nasal polyps. One major outcomes research study (level II) and more than a hundred reviewed case series (level IV) with highly consistent results suggest that patients with CRS with and without polyps benefit from sinus surgery. Major complications occur in less than 1%, and revision surgery is performed in approximately 10% within 3 years. However, in the majority of CRS patients, appropriate medical treatment is as effective as surgical treatment. Sinus surgery should be reserved for patients who do not satisfactorily respond to medical treatment (level 1b). Functional endoscopic surgery is superior to minimal conventional procedures including polypectomy and antral irrigations (level Ib), but

Table 3. Treatment evidence and recommendations for adults with chronic rhinosinusitis with nasal polyps. *

Therapy	**level**	**grade of recommendation**	**relevance**
oral antibiotics short term < 2 weeks	no data	D	no
oral antibiotic long term > 12 weeks	Ib	A	yes, for late relapse
topical antibiotics	no data	D	no
topical steroids [55, 56]	Ib	A	yes
oral steroids [57, 58]	Ib	A	yes
nasal douche	Ib, no data in single use	A	yes, for symptomatic relief
decongestant topical/oral	no data in single use	D	no
mucolytics	no data	D	no
antimycotics – systemic [59]	Ib (-)#	D	no
antimycotics – topical [52, 53]	Ib (-)	A	no
oral antihistamine in allegic patients [59]	Ib (1)	A	yes, in allergy
capsaicin [60]	II	B	no
proton pump inhibitors [61]	II	C	no
furosemide [62]	II	C	no
immunomodulators	no data	D	no
phytotherapy	no data	D	no
anti-leukotrienes [63, 64]	III	C	no

* Some of these studies also included patients with CRS without nasal polyps

: Ib(-) study with a negative outcome

superiority to inferior meatal antrostomy or conventional sphenoethmoidectomy is not yet proven.

In CRS patients not previously operated, extended surgery does not yield better results than limited surgical procedures (level Ib). Although not evidence-based, the extent of surgery is frequently tailored to the extent of disease, which appears to be a reasonable approach. In primary paranasal surgery, surgical conservatism is recommended.

Revision endonasal sinus surgery is only indicated, if medical treatment is not sufficiently effective. Substantial symptomatic improvement is generally observed in both, CRS with and without polyps, though the improvement is somewhat less than after primary surgery. Complication rates and particularly the risk of disease recurrence are higher than after primary surgery.

EVIDENCE BASED MANAGEMENT SCHEME FOR ADULTS WITH ACUTE RHINOSINUSITIS FOR ENT SPECIALISTS

Diagnosis

Sudden onset of rhinosinusitis symptoms as described above with signs of inflammation using nasal endoscopy and exclusion of dental infection. A plain X-ray is not recommended and CT-Scan is also not recommended unless additional problems exist such as very severe disease, immunocompromised patients or signs of complications.

EVIDENCE BASED MANAGEMENT SCHEME FOR ADULTS WITH CHRONIC RHINOSINUSITIS WITHOUT NP FOR OTORHINOLARYNGOLOGISTS

Diagnosis

Symptoms as described above present longer than 12 weeks. The severity of symptoms should be assessed because it is relevant for the choice of treatment [11]. Nasal endosocpy can be completed if necessary with imaging [21], culture [22], mucociliary clearance testing [23], allergy testing, nasal airway assessment [24, 25], testing of olfaction [26, 27] and/or signs of immunodeficiencies. Attention should be given to the lower airways [28] QOL measurements can be relevant especially for evaluation of treatment [29-31].

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REVIEW

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Observations on the ability of the nose to warm and humidify inspired air*

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SUMMARY

The major function of the nose is to warm and humidify air before it reaches to the lungs for gas exchange. Conditioning of inspired air is achieved through evaporation of water from the epithelial surface. The continuous need to condition air leads to a hyperosmolar environment on the surface of the epithelium. As ventilation increases, the hyperosmolar surface moves more distally, covering a larger surface area of the airway, and stimulates epithelial cells to release mediators that lead to inflammation. This inflammation is not identical to allergic inflammation, but causes both short-term and long-term changes in the epithelium. In the short term, it increases paracellular water transport in an attempt to enhance conditioning, and it stimulates sensory nerves to initiate neural reflexes. It also disrupts channels in the cellular membrane, which might permit greater penetration of foreign proteins, such as allergens, leading to further inflammatory cascades. The long-term inflammation induced over time by the hyperosmolar milieu could worsen the ability of the nose to condition air, requiring more of the conditioning to occur in the lower airway and leading to adverse consequences for the respiratory system.

Key words: humidity, nose, allergic rhinitis, temperature, water transport

Adults condition more than 14,000 liters of air per day; this requires more than 680 grams of water, approximately 1/5 of our adult daily water intake [1]. The mechanisms, by which the nose conditions inspired air and how this ability is altered in patients with allergic rhinitis and asthma, are the subject of this review.

Water transport: a fundamental biological process

The regulation of the transport of water across biological membranes is fundamental to the maintenance of homeostasis between bodily fluid compartments, to the preservation of organisms under adverse conditions, and, indeed, to life itself [2]. Higher organisms could not exist without epithelial barriers that separate the internal and external milieus. To separate these compartments, cell membranes are composed of lipid bilayers that are relatively impermeable to ions. Therefore, to facilitate biological processes, ions must cross these membranes or pass between cells to exert their effects.

Membrane proteins known as ion channels, pumps, and transporters mediate water transport. Ion channels enable rapid passive movement of selected ions across cell membranes. More than 100 families of channel-forming proteins/peptides exist in prokaryotes and eukaryotes [3]. Transcellular transport through specific membrane pumps and channels actively generates electro-osmotic gradients that are critical for a variety of cellular functions [4]. Tight junctions, located between cells, are the main routes for passive ion permeation. Inflammatory mediators, such as histamine, can alter tight junctions, allowing macromolecules to pass from the external to the internal environment [5, 6].

Aquaporins and channelopathies

Besides the classic Na^+ and Cl^- ion transporters, other proteins can be involved in water transport, such as the glucose transporter, the c-AMP-activated cystic fibrosis transmembrane conductance regulator, the urea transporter UT3, and multiple Na^+ -solute cotransporters. Aquaporins (AQs), a family of small membrane-spanning proteins, are expressed in plasma membranes of many cell types involved in fluid transport [7]. The expression of many AQs is functionally significant for movement of water across cell membranes. Interestingly, they respond to osmotic gradients, and their activity is generally measured by an osmotic swelling assay [8]. Mutation in the AQP2 water channel causes the rare non-X-linked form of hereditary nephrogenic diabetes insipidus [9] and shows the requirement of the human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine [9]. Aquaporins have been implicated in respiratory disease. For example, AQP1, -4, and -5 are expressed in lung tissue. Transgenic aqua-

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porin knockout mice with targeted gene disruption in AQP1 and AQP5 have very low lung water permeability [2]. Additionally, AQP-5-deficient mice have been shown to show bronchial hyperreactivity [10]. These data implicate water transport in related respiratory disease in the lower airway, which may also characterize the upper airway.

Channelopathies, diseases that result from defects in ion channel function, are being discovered with increasing frequency. Channelopathies arise through a number of mechanisms, such as mutations in the promoter and coding region of ion channel genes, defects in genes encoding molecules that regulate channel function, or the development of autoantibodies to channel proteins that inhibit their function. Additionally, many drugs and mediators such as phosphodiesterase inhibitors, nitric oxide, VIP, and leukotrienes have effects on ion channels, affording another mechanism by which they can develop acquired or secondary dysfunctions that can cause disease [11]. Many diseases also have secondary effects on ion channel activity, for example, maturity-onset diabetes. Hence, the role of water transport proteins and ion channels is relevant to a number of diseases through a wide variety of mechanisms.

The epithelial barrier function and beyond

There is growing evidence that the respiratory epithelium has a number of functions in addition to its role as a barrier between the internal and external environments. It produces multiple cytokines that participate in airway inflammation, such as granulocyte macrophage colony-stimulating factor, for which the epithelium is the principal source [12]. The epithelium also makes metalloproteases that may be involved in airway remodeling [13]. Holgate hypothesized that a primary defect in the epithelium, which causes abnormal responses to various stimuli and cannot undergo the normal repair process, is responsible for asthma [14]. The epithelium is also now recognized as a critical component of the innate immune system.

Epithelial defects may be secondary to chronic inflammation. To illustrate this point, an analogy can be drawn to inflammatory bowel disease. In the gut of patients with this disorder, inflammation affects water transport and leads to diarrhea [15]. For years, the mechanism postulated to underlie the diarrhea was an inflammation-induced increase in secretions. We now know that the diarrhea is actually caused by increased production of interferon, which diminishes absorption of Na^+. This is an example of the interaction between inflammation and epithelial water/ion transport that can cause disease.

Another component of epithelial function is nasal mucociliary transport, an important factor in heat and water exchange and protection of the mucosal interface. This process requires an aqueous periciliary fluid layer of a height that allows cilia to move the viscoelastic mucus on its surface. Too much or too little periciliary fluid leads to ineffective mucociliary transport, which can lead to disease. For example, dryness leads to increased bacterial adherence and is believed to play a role in the development of sinusitis.

How might these processes be affected to cause disease in the upper and lower airway? A number of studies have suggested that decreased water transport in the upper airway causes conditioning to occur lower in the airway. McFadden and colleagues showed that air not fully conditioned by the nose will have to be conditioned further by the lower airway [16]. Annensi et al. showed that subjects reporting nasal sensitivity to cold dry air (CDA) had a more rapid decline in FEV_1 over five years compared to those without such sensitivity [17]. Inhalation of the same volume of dry air through the mouth, in contrast to the oronasal route, causes a greater reduction in FEV_1 in asthmatic subjects [18, 19]. Moreover, prolonged repeated exposure of the airways to inadequately conditioned air can induce inflammation in the lower airways [20], the penultimate example being the changes that occur in the trachea after a total laryngectomy.

Dehydration injury of the epithelium includes epithelial desquamation, leukocyte infiltration, vascular leakage, and mast cell degranulation, all of which can worsen inflammation. Furthermore, a change of the epithelium from ciliated to squamous nonciliated leads to a further decrease in its ability to transport water. Hence, the study of nasal conditioning has both a fundamental basis in the critical function of water transport, an important relationship to inflammation, and direct clinical relevance to a variety of diseases, including those of the upper and lower airway.

Models of nasal conditioning

We have been interested in understanding nasal function in health and disease. Toward this goal, we have developed several *in vivo*, human models of nasal function. We have consistently used the relevant organ in the relevant species to address questions about the mechanisms that underlie nasal air conditioning.

Nasal provocation with cold, dry air

We were interested in studying the mechanism by which the inhalation of cold, dry air induces rhinorrhea. We reasoned that the inhalation of dry air caused drying of the nasal mucosa and creation of a hyperosmolar milieu, which can activate mast cells *in vitro*, leading to mediator release and subsequent symptoms [21].

We thus allowed subjects to breathe CDA and monitored the subsequent response by scoring symptoms and measuring the levels of mediators in nasal lavage. CDA resulted in an increase in symptoms compared to baseline and in the release of inflammatory mediators. The pattern of these recovered mediators suggests that mast cells participate in this nasal reac-

tion. Because the early response to CDA produces the same pattern of mediator release, as does the early response to antigen, we asked whether a late-phase reaction follows the response to CDA. In fact, significantly more symptoms and higher histamine and TAME levels (a marker of vascular permeability) than in control exposures were recovered in the first ten hours after CDA, showing a late inflammatory response [22]. Additionally, epithelial cells in the lavage fluid were found in increased numbers compared to those in appropriate controls, suggesting that tight junctions are disrupted [23].

We then undertook several studies to establish a mechanism for mast cell activation. To address the hypothesis that the hyperosmolar milieu generated by the drying of the nasal mucosa stimulated the release of mast cell mediator, we followed two directions: we evaluated the effect of nasal mucosal provocation with a hyperosmolar stimulus, and we attempted to determine changes in the osmolality of surface secretions after CDA challenge.

Healthy human volunteers underwent nasal challenge with isosmolar and hyperosmolar mannitol solutions. We found that hyperosmolar challenge caused histamine and leukotriene C4 release [24]. Dose-response curves between increasing osmotic loads and histamine recovery in lavage fluids were obtained. We concluded that hyperosmolar stimuli cause histamine release in vivo, possibly from mast cells.

Although a spectrum of responsiveness to CDA probably exists in the general population, we were able to select both individuals who respond and those who do not respond to the CDA challenge, based on the presence or absence of a typical history of nasal symptoms upon exposure to a cold and windy environment. This criterion has a specificity of 94% in selecting a CDA responder. To assess whether the reactivity to hypertonic loads of the two extreme groups differs, we challenged 11 CDA responders and 19 non-responders with isosmolar and hyperosmolar mannitol solutions [24]. The results indicated that CDA responders released more histamine in their nasal secretions after hyperosmolar provocation than did CDA non-responders, possibly because of impairment of water transportation across the mucosa.

The second approach to linking hyperosmolarity to the CDA-induced response involved measurement of the osmolarity of nasal secretions after CDA challenges. We initially measured the osmolality of returned lavage fluids (25). In each of 9 CDA responders, this index was increased after CDA challenge, compared to baseline, from 288 ± 3 to 306 ± 5 mOsm/kg H_2O ($p < 0.01$). In contrast, the returned-fluid osmolality of six CDA non-responders did not differ from baseline. Significant correlations were found between mediator concentrations and the osmolality of recovered lavages ($rs = 0.617$, $p < 0.02$; $r_s = 0.679$, $p < 0.01$ for histamine and TAME, respectively). As a control, we measured the osmolarity of nasal secretions obtained after allergen challenge of atopic individuals and found no significant changes. These studies provided the first evidence in human subjects that inhalation of CDA increased the osmolality of respiratory secretions. Although the changes were statistically significant and different from those in appropriate controls, the increments in osmolality were small, most likely secondary to the dilutional effect of the isosmolar saline lavage used for collecting secretions. We sought, therefore, to measure the osmolality of surface secretions directly.

We collected secretions directly from the mucosas of CDA-sensitive individuals with filter paper discs before and after challenge. The limitation of this method was that, except on rare occasions, we could not obtain a sufficient volume of nasal secretions at baseline to perform osmolality measurements. We therefore chose to compare the osmolality of CDA-induced secretions to that of methacholine- and histamine-induced secretions. Because CDA non-responders have little or no secretion on their mucosal surface after CDA challenge, only CDA responders were evaluated with these protocols [25]. The osmolality of nasal secretions (mOsm/kg H_2O) (mean ± SEM; $n = 8$) after provocation with CDA was 381 ± 5.6; with methacholine, 337 ± 3.5; and with histamine, 315 ± 3.1. Histamine, which, in addition to glandular stimulation, induces vascular permeability, resulted in the lowest osmolality. In contrast, methacholine, a glandular secretagogue, produced slightly hyperosmolar secretions. Cold, dry air led to significantly higher osmolality compared to either methacholine or histamine ($p < 0.05$), confirming our hypothesis that the osmolality of nasal secretions is increased after inhalation of CDA. These data also suggest that nasal glandular secretions are hyperosmolar. This finding is in agreement with the data of Mann and colleagues in dogs [26]. More importantly, these combined observations were consistent with our overall hypothesis that individuals vary in their ability to condition air, and those with the least ability to condition air develop hyperosmolar secretions and a clinical response to CDA inhalation.

The model used in the above experiments involves the inhalation of air through the nose and exhalation through the mouth. Strohl and colleagues showed that the inhalation of air through the nose and exhalation through the mouth induced an increase in nasal airway resistance, but when the same subjects inhaled and exhaled air through the nose, their airway resistance did not increase [27]. They interpreted their experiment to imply that the pattern of breathing influences the response, and that the recovery of heat during expiration prevents the response. This work appeared to negate our studies.

To address this concern, we performed experiments in which we assessed the response of subjects to CDA when it was both inhaled and exhaled through the nasal cavity [28]. In contrast to Strohl, we performed our experiments in 10 subjects who gave

a history of clinical sensitivity to cold, windy environments and who had previously responded to our standard CDA challenge. The subjects were randomized to breathe either CDA or warm moist air (WMA) in and out through the nose for 45 minutes during two separate visits. The total change in secretion weight from baseline after the CDA exposure was 30 ±10 mg compared to 0 ± 1 mg for WMA, the difference being highly significant ($p < 0.009$). During the WMA challenge, the levels of histamine and TAME esterase did not change significantly from baseline. In contrast, after breathing of CDA, there was a significant increase ($p < 0.01$) from baseline in the levels of both histamine (3.9 ± 1.2 to 10.6 ± 2.7 ng/ml) and TAME (3.8 ± 1.4 to 4.6 ± 1.6 cpm). Although significantly increased, these levels did not change to the extent of those reported previously when the subjects inhaled the CDA through the nose and exhaled it through the mouth. This difference was anticipated based on the reduction of the stimulus, the amount of air to be conditioned. The fact that there was a significant change implies that the nasal mucosa does respond to conditioning CDA even though there is an estimated 30% recovery during exhalation. We believe that our protocol of breathing in through the nose and out through the mouth represents a means to augment the stimulus so that it is easier to study it. An analogy is that the inhalation of air with 5% CO_2 at 140 liters through the mouth while seated wearing nose clips serves as a model of exercise-induced asthma.

We then switched our focus from studying the mechanism of the CDA response to measuring the mechanics of the ability of the nose to condition air by using a nasal probe as our model.

Development of a nasal probe

The nose functions to warm and humidify air from ambient conditions that range from temperatures of -42 to 48°C and relative humidities from 0 to 100% [29]. Nasal conditioning occurs from a resting ventilation of approximately 5 liters per minute (l/min) to sustained flow rates of 20 to 30 l/min before nasal breathing is supplemented with oral breathing. We reasoned that, if we could measure the temperature and relative humidity of inhaled air at the nasal inlet and then in the nasopharynx, we would be able to calculate the water content of the air at these two locations. The posterior (nasopharynx) measurements sample the airstream immediately after it exits the nose, thus providing information regarding the end results of nasal function. The difference between these contents (nasal inlet and nasopharynx) represents the amount of water invested by the nose into inhaled air, a good reflection of the conditioning capacity of the nose. Furthermore, because there is strong evidence that exhaled air is fully saturated, it would be necessary only to measure conditioning after inhalation [29-32]. We therefore developed a probe for measuring the temperature and humidity of inhaled air within the nasopharynx and a similar one for measuring the same parameters at the nasal inlet.

In a typical experiment, one of the patient's nostrils was decongested and anesthetized with oxymetazoline and lidocaine, and the probe was inserted through that nostril and positioned such that the tip of the probe bearing the temperature and humidity sensors was suspended in the nasopharynx, sampling air exiting the nasal cavities. This was confirmed by nasal endoscopy. That nostril was then occluded with a wax plug, and the other nasal cavity was exposed to air of different temperatures and humidities via a mask applied over the nose. The patients were instructed to breathe through their mouth while air was blown continuously and unidirectionally through the nose at flow rates of 5, 10, and 20 l/min. The temperature and humidity of the inhaled air were continuously sampled via a similar sensor placed in the mask at the inlet of the nasal cavity. This experimental design permitted the development of steady-state conditions that were easily measurable by the probe and circumvented the potential problems associated with exhalation.

The nostril used as a conduit for the probe was not studied, because pre-medication of that nostril (to facilitate insertion of the probe) as well as probe-induced distortions of airflow patterns within the nostril could interfere with the conditioning function of that nostril. Thus, sensors were placed at the nasal inlet and in the nasopharynx, and they sampled air entering and exiting the open, non-manipulated nostril, allowing us to evaluate the conditioning capacity of that nostril. The range of flow rates from 5 to 20 l/min spans flows at rest to values at which most individuals switch from nasal to oronasal breathing. It should also be noted that air blown unidirectionally prevents air exiting from the lung at 37°C and 100% RH from condensing on the probe and interfering with sampling in the nasopharynx. The duration of air sampling in the mask and nasopharynx was 22 min, with data collected only during the last 15 min of each challenge used for analysis. The initial 7-minute period of each challenge was disregarded because it reflects the time necessary for the temperature in the nasopharynx to reach a steady state.

To ensure that the probe sensors retained an adequate response time when positioned in the nasopharynx, the subjects forcefully inhaled room air through the nose with the mouth closed. This maneuver created an airflow transiently exceeding 100 l/min. A rapid change in both temperature and RH readings during the sniffs reflected adequate calibration and response times for the studies presented here. Subjects were asked to breathe room air and perform the "sniff" test periodically during the experiments to ensure proper functioning of the probe.

The nasopharyngeal temperatures at 5, 10, and 20 l/min for all subjects during exposure to CDA were 33.4 ± 0.7°C, 30.5 ± 1.1°C, and 25.9 ± 1.4°C, respectively. The temperature fell sig-

nificantly with increasing flow rates ($p = 0.0001$). Post-hoc analysis of the nasopharyngeal temperatures obtained at different flow rates showed a statistically significant difference between the temperatures obtained at 5 l/min compared to those obtained at both 10 and 20 l/min ($p < 0.05$), as well as a significant difference between temperatures obtained at 10 l/min compared to 20 l/min ($p < 0.05$). Furthermore, nasopharyngeal temperatures during exposure to CDA were consistently lower than those during exposure to WMA at each flow rate ($p < 0.01$).

Individuals showed a wide variability in their ability to condition air, but the RH of air in the nasopharynx was consistently at 100%, irrespective of the temperature and RH of the inhaled air. There was no correlation among nasal airway resistance or nasal volume obtained prior to challenge, nasopharyngeal temperature, and the slight variability in body temperature. Proctor, in discussing the wide variability among individuals, speculated that prior viral infections may have altered the epithelium, thus producing the variability [29]. As mentioned below, we think that heritability plays a role in this variability.

Because the temperature and RH of inhaled air as well as air exiting the nasal cavity into the nasopharynx were known, we were able to calculate their respective water contents and subsequently the water gradient (WG) between inhaled and conditioned air. The WG represents the amount of water invested by the nasal mucosa to condition inspired air. After exposure to CDA, the WG at 5, 10, and 20 l/min. was 297.6 ± 12.6 mg, 509.1 ± 32.0 mg, and 794.1 ± 62.1 mg, respectively, showing a statistically significant increase in the amount of water generated by the nose for conditioning inspired air with increasing air flow rates. The reproducibility of the nasal response to conditioning CDA was studied in 8 nonallergic subjects on 3 separate visits. During all three visits, there were flow-dependent significant increases in the water gradient across the nose. After exposure to CDA the difference in mean total water gradient (TWG) values for the three visits was not statistically significant ($p = 0.56$). The coefficient of variation in % (standard deviation/mean x 100%) of the TWG obtained during the three visits ranged between 5.1% and 33.8% and averaged 14.7%.

Once we were confident in our ability to measure the conditioning capacity of the nose in a reliable and reproducible fashion, we turned our attention to investigating factors that might influence this conditioning capacity by contributing to the amount of water delivered to inspired air. Among these, the geometry of the nasal cavity and the temperature of the nasal mucosa appear to be key [33]. Therefore, we first studied the effects of raising the nasal mucosal surface temperature by immersion of the feet in warm water. This technique was based on observations in 1954 by Cole, who showed that the nasal mucosal temperature rose approximately 2°C when a fan blew heat from an open flame onto the dorsal skin of subjects [34]. This increase occurred without a concurrent increase in the body core temperature.

Studies of the microcirculation of skin and its contribution to heat exchange predict that the increase in nasal mucosal temperature after external thermal stimulation is secondary to a neural reflex [35]. Our method of heating the feet by immersion in a warm water bath reproduced that of Cole. Six subjects were randomized to immersion of the feet in 30°C and then 40°C water, and their nasal mucosal temperature was measured by gentle application of the temperature sensor against the nasal mucosa (36). The nasal mucosal temperature increased significantly, to 32.2 ± 1.3°C after immersion of feet in 30°C water and to 33.1 ± 1.2°C after immersion of feet in 40°C water ($p < 0.05$). There was no concomitant change in nasal volume, as measured by acoustic rhinometry, between the two exposure groups (30°; 17.8 ± 4.5 cc; 40°; 17.7 ± 5.3 cc). There was a significant increase in the conditioning capacity of the nose in response to cold-air challenge during the 40°C immersion (1669 ± 312 mg water) when compared to the 30°C immersion (1324 ± 152 mg water) ($p < 0.05$). From these data, we deduced that warming the nasal mucosa improves the ability of the nose to condition inspired air without a concomitant change in the volume of the nasal cavity. These findings are consistent with the theoretical model of heat and water vapor transport across the nose developed by Hanna, and they support the accuracy of our setup for measuring nasal air conditioning [37].

Effect of allergic inflammation on the ability of the nose to condition inspired air

After establishing our ability to measure nasal conditioning, we studied the effect of allergic inflammation on that function. In prior studies, asymptomatic subjects with seasonal allergic rhinitis outside their season showed no alteration in their nasal functions and in their indices of inflammation when compared to normal subjects. Therefore, we first compared the nasal conditioning capacity of these 2 groups. The response to inhalation of CDA was compared between 11 nonallergic subjects and 22 allergic subjects out of season. Allergic subjects had significantly lower nasopharyngeal temperatures than did nonallergic subjects at 5 l/min (31.7 ± 0.5°C vs 34.6 ± 0.9°C, $p = 0.0004$) and 10 l/min (28.2 ± 0.5°C vs 32.2 ± 1.5°C, $p = 0.003$). Comparing allergic to nonallergic subjects, there was a significant difference in WG values obtained at 5 and 10 l/min as well as in the TWG (Figure 1). The reason for the difference was not apparent.

To examine the effect of allergic inflammation, we studied the ability of seasonal allergic subjects to condition air in and out of their allergy season. We selected 10 individuals with seasonal allergic rhinitis and measured their ability to warm and humidify air before the ragweed season and then slightly past

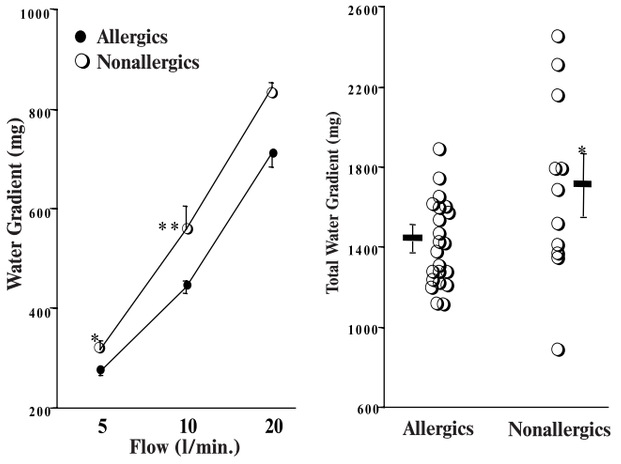

Figure 1. Comparison of the response to CDA exposure of allergic subjects out of season (n=22) and nonallergic (n=11) subjects. The left panel demonstrates the mean ± SEM of the water gradient across the nose at each of the exposure flow rates used. Closed circles represent the response of the allergic subjects and open circles that of the nonallergic subjects. The right panel depicts the total water gradient across the nasal cavity for all three flow rates of CDA. Solid bars represent mean ± SEM of the individual data points. *$p < 0.05$ and **$p < 0.01$ vs allergic subjects.

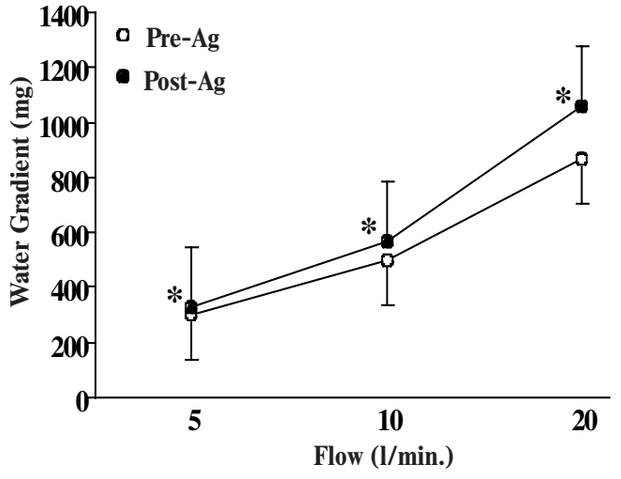

Figure 2. Comparison of the response to CDA exposure of allergic subjects out of season (n=20) before (open circles) and 24 hours after (closed circles) allergen challenge. The graph depicts mean±SEM of water gradient expressed in mg across the nose at each of the exposure flow rates used. *$p < 0.05$ vs pre-antigen.

the pollen peak. The TWGs before vs. during the season were 1523 ± 315 vs. 2050 ± 438 mg ($p < 0.01$)[36]. Therefore, the presence of allergic inflammation improved the ability of the nose to condition air.

For better control of the factors that could be responsible for this change, we initiated a trial involving nasal challenge with antigen [38]. Twenty subjects with seasonal allergic rhinitis were investigated outside their season. We measured their ability to condition air before and 24 hours after challenge. We quantitated the degree of inflammation by counting eosinophils, measuring the level of albumin in nasal lavages, and recording symptoms. Twenty-four hours after allergen challenge, there was an increase in the number of eosinophils and in the level of albumin in recovered nasal lavages. As in the seasonal study, allergic inflammation increased the ability of the nose to condition inspired air (Figure 2). There was no significant relationship between the indices of allergic inflammation that we assessed and the change in conditioning capacity.

Changing nasal volume

Allergic rhinitis is consistently associated with nasal congestion, which results from pooling of blood in the cavernous sinusoids and a subsequent decrease in nasal volume. Therefore, an allergen-induced increase in nasal congestion seems like a logical explanation of the increase in the conditioning capacity of the nose observed in allergic inflammation.

We tested the hypothesis that increasing nasal congestion improves nasal air conditioning. We performed a randomized, 2-way crossover study on 6 healthy subjects to investigate the effect of decreased nasal volume, induced by placement of subjects in the supine position, on the conditioning capacity of the nose [39]. Subjects underwent nasal conditioning measurement in both upright and supine positions at each visit. The order of which position was first and which was second was randomly assigned, and, on the second visit, the order was reversed. The same technique as detailed above was used for measurement of the conditioning capacity of the nose in response to a CDA stimulus, and acoustic rhinometry was used for assessment of nasal patency. The nasal volume decreased significantly from baseline without a change in the mucosal temperature when subjects were placed in the supine position ($p < 0.01$). The TWG in the supine position was significantly lower than that in the upright position ($p < 0.001$) (Figure 3). There were no significant differences in the percentages of CDA-induced decrease in the nasal volume between the two positions ($p = 0.5$). In the supine position, however, the nasal mucosal temperature after CDA exposure was significantly lower than that in the upright position ($p < 0.01$). Our data showed that placing a subject in the supine position decreased the ability of the nose to condition CDA compared to that in the upright position. We speculate that the decreased nasal conditioning capacity in the supine position is related to the decrease in nasal mucosal temperature induced by an increase in air pressure and speed.

According to a theoretical model of localized heat and water vapor transport in the nose, the two most important parameters predicting the air-conditioning process are the nasal mucosal temperature and the volume of the nasal cavity [37]. We reduced the nasal volume without altering the mucosal

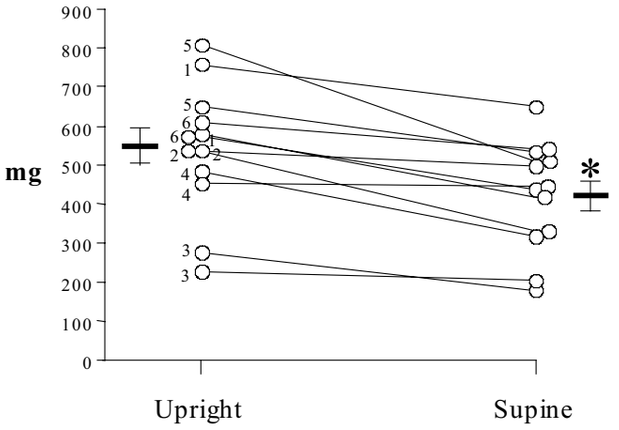

Figure 3. Individual data of total water gradient expressed in mg across the nose after placing subjects in 2 different positions as specified on the abscissa. The thick bars represent mean ± SEM of the individual data points. Individual subjects have been numbered to show reproducibility of measurement. **$p < 0.001$ vs. Upright.

temperature by placing subjects in the supine position and studied this effect on the nasal conditioning capacity. Contrary to the theoretical model, subjects were less able to condition CDA in the supine position, compared with the upright position, demonstrating the need to test the theoretical models with human data, for the simple prediction from a theoretical model did not account for the complexity of the human situation. The study also supports the clinical practice of elevating the head of the bed of recovering head and neck surgery patients.

Temperature elevation

We have previously shown that raising the nasal mucosal temperature by immersing feet in warm water increases the amount of water evaporated by the nose as air passes through it (nasal conditioning capacity). To investigate further the effect of nasal mucosal temperature on the nasal conditioning capacity, we raised the temperature through α-adrenoreceptor blockade by intranasally administering phenoxybenzamine. We hypothesized that blocking α-adrenoreceptors during inhalation of CDA would lead to an increase in nasal blood flow, surface temperature, and nasal conditioning capacity, as measured by the WG. After appropriate pilot studies, we performed a double-blind, placebo-controlled, 2-way cross-over study on 9 non-atopic, healthy subjects by studying the effect of treatment with intranasal phenoxybenzamine [40]. The nasal mucosal temperature increased significantly after administration of phenoxybenzamine. This increase was associated with a significantly smaller net decrease in nasal mucosal temperature after exposure to CDA ($p < 0.05$). However, there were no significant differences in nasal conditioning capacity between treatments ($p > 0.05$) (Figure 4). Phenoxybenzamine decreased the symptom of rhinorrhea after exposure to CDA ($p < 0.05$), but congestion did not differ between individuals given phenoxybenzamine and those given placebo ($p > 0.05$). Our data demonstrate that phenoxybenzamine, despite raising the mucosal temperature and not affecting the nasal volume, did not affect the ability of the nose to warm and humidify air.

Influence of glandular secretions

Major contributors to the volume of surface secretions are the parasympathetically driven glands in the nose [41]. Blocking of the parasympathetic system with anticholinergic agents reduces rhinorrhea [42, 43]. Ipratropium bromide is a commercially available anticholinergic agent for treatment for excessive rhinorrhea. Although ipratropium bromide treats the rhinorrhea appropriately, we were concerned that it might worsen the ability of the nose to condition air. To address this issue, we performed a double-blind, placebo-controlled study involving 15 normal subjects [44]. The subjects were pretreated with either ipratropium bromide (0.06%) or normal saline sprayed into the nasal cavity and then underwent challenge with three increasing flows of CDA. We evaluated not only the effect of ipratropium on nasal conditioning, but also its effects on nasal

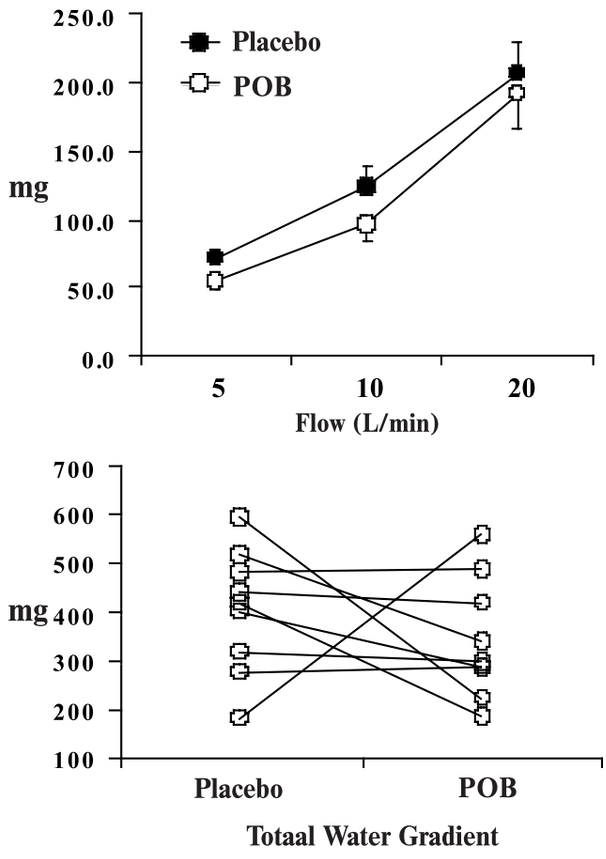

Figure 4. Effect of phenoxybenzamine on nasal conditioning capacity. Top panel: Water gradient (mg) across the nose at three flow rates (5, 10, 20 l/min). Data are mean ± SEM for 9 subjects. There was increased conditioning with increasing flow rate. There were no differences between treatments. Bottom panel: Individual data of total water gradient (mg) across the nose. POB = phenoxybenzamine.

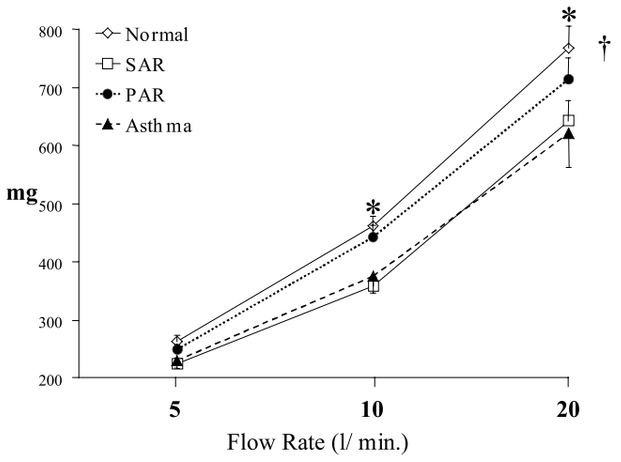

Figure 5. Water gradient across the nose in each group of subjects at 3 flow rates (5, 10, and 20 l/ min.). Data are mean ± SEM for 15 subjects in each group as shown by symbols. SAR: seasonal allergic rhinitis. PAR: perennial allergic rhinitis. *$p < 0.001$ vs. respective 5 l/min in all groups, †$p < 0.001$ vs. respective 10 l/min in all groups.

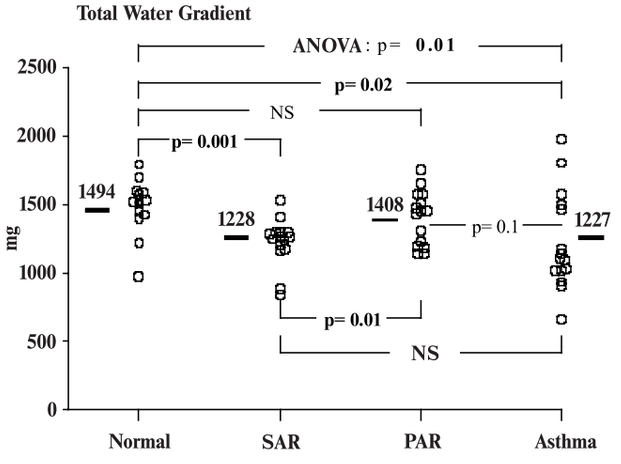

Figure 6. Individual data of total water gradient across the nose in each group of subjects specified on the abscissa. The solid bars with the numbers above them represent the mean of the individual data points; NS: not significant.

symptoms, nasal volume, and changes in the albumin and osmolality of the lavage fluid. Ipratropium bromide improved the conditioning of inspired air, as demonstrated by enhancement of the water supplied to the inhaled air during its passage across the nasal cavity. The TWG was 2432 ± 152 mg after placebo and 2926 ± 149 mg after ipratropium bromide ($p < 0.01$). The nasal volume decreased after exposure to CDA inhalation when the patients were pretreated with saline (7.85 to 4.29 cc, $p < 0.001$). The decrease after treatment with ipratropium bromide was also significant (from 6.89 to 3.88 cc), but the net decrease was significantly less after ipratropium bromide ($p = 0.01$) compared to saline premedication, although the difference was small. The increase in secretions after exposure to cold, dry air compared to baseline was significantly less (7.1 to 21.2 mg vs 2.32 to 11.01 mg, $p < 0.05$) after ipratropium pretreatment than after pretreatment with saline. Albumin levels were greater on the days when the patients received ipratropium, suggesting increased vascular permeability.

The changes in the symptoms of rhinorrhea and nasal congestion paralleled the objective measurements. Nasal secretion osmolality increased following CDA exposure after both treatments, but the magnitude of the increase was reduced after ipratropium, a finding consistent with the observation by Mann et al. that glands induce hyperosmolar secretions [26].

To investigate whether the ipratropium-induced increased conditioning capacity of the nose was related to an effect on the nasal mucosal temperature, we conducted another series of experiments in which 7 normal subjects were premedicated in a double-blinded manner with either intranasal saline or ipratropium bromide (0.06%). The subjects were then exposed to CDA at 20 l/min. The nasal mucosal temperature was measured before application of the medication, after drug administration, and after 8 minutes of exposure to CDA at 20 l/min. Pretreatment with ipratropium did not lead to any changes in nasal mucosal temperature, and breathing CDA resulted in lowering of the nasal mucosal temperature to identical degrees after premedication with ipratropium and saline.

This study clearly demonstrates that blocking of the glands of normal individuals does not impair their ability to warm and humidify inspired air, a clinically useful observation. However, it points to the complexity of the nasal response in the face of altering of one parameter. We believe that the explanation for our data lies in the increased delivery of heat to the surface caused by an increase in blood flow secondary to the nasal mucosal response to conditioning air [44].

Our data on the allergic state may seem conflicting. First, atopy without inflammation is associated with a decreased ability to condition air. This suggests that either a primary or a secondary defect (associated with years of allergic inflammation) in water transport is associated with atopy. The second issue relates to the increased ability of atopic individuals to condition air when they have ongoing allergic inflammation. The precise reason for this increased ability to condition air is not apparent. It can relate to the effects of mediators released during allergic inflammation that have an impact on water transport mechanisms. Physiologic changes in blood flow and reactivity of nerves could also play a role.

Nasal conditioning in asthma

We showed above that seasonal allergic individuals had a reduced ability to condition air, which was improved by inflammation. We hypothesized that individuals with perennial

allergic rhinitis (PAR), who had ongoing inflammation, would condition air in the same way as do seasonal allergic subjects with inflammation. Because individuals with asthma usually have allergic inflammation in both the nose and the lungs, we hypothesized that they would have the ability to condition air nasally like individuals with PAR. We performed a prospective, parallel study in 15 normal subjects, 15 subjects with seasonal allergic rhinitis (SAR) outside their allergy season, 15 subjects with PAR, and 15 asthmatic subjects [45]. We measured the ability of the noses of these subjects to humidify CDA. The TWG in the SAR group was significantly lower than that in normal subjects (Figures 5 and 6). There were no significant differences in TWG between the PAR and normal groups. Contrary to our hypothesis, asthmatic subjects had a significantly lower TWG than did normal subjects. There was a significant negative correlation between TWG and Aas score (which is a reflection of the severity of asthma) in the asthmatic group ($rs = -0.8$, $p = 0.0007$).

Our data show that asthmatic subjects have a reduced ability of the nose to condition CDA. The mechanism underlying the observed differences in nasal conditioning among the groups above did not involve nasal volume, surface temperature, or glandular reactivity. We speculate that the reduced conditioning capacity of the nose may adversely affect the lower airway.

Because asthmatic subjects have inflamed airways that respond to steroids, we speculated that treating the inflammation would worsen their ability to condition inspired air. This speculation was based on our previous data showing that allergic inflammation improved the nasal conditioning of the nose. We performed a double-blind, placebo-controlled study investigating the effects of budesonide on nasal conditioning (Figure 7) [46]. Consistent with our hypothesis, the intranasal steroid reduced the ability of 9 of 10 asthmatic subjects to condition air; i.e., reducing inflammation made the defect in water transport more apparent. This observation is consistent with our findings on the effects of natural and induced allergic inflammation on nasal air conditioning. This observation might explain some of the clinically observed, local adverse effects of intranasal steroids, drying, and local irritation.

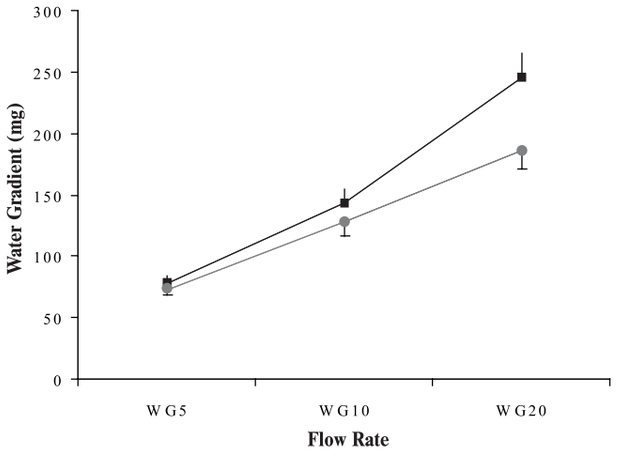

Figure 7. Response to nasal conditioning of 10 asthmatic subjects treated for 1 week with placebo (■) or budesonide (●).

Heredity and nasal conditioning capacity

In our previous studies on nasal conditioning, we observed a large variability among individuals in their ability to condition inspired air. Although we previously investigated different parameters such as age, sex, nasal mucosal temperature, pulse, blood pressure, and nasal volume, we have been unable to explain this variability. We hypothesized that heredity contributes to the differences in the nasal conditioning capacity of individuals. To address this hypothesis, we performed a prospective study on 47 sibling pairs. Cold, dry air was delivered to the nose, and we calculated the TWG to find the nasal conditioning capacity. We found a highly significant intraclass correlation of 0.53 ($p < 0.0001$) between sibling pairs for the TWG. These results suggest that there is a genetic basis for nasal conditioning, and they point to the possibility that a genetic variability in expression, or lack thereof, of one of the proteins described in the introduction of this review may be responsible for our observations.

CONCLUSION

In conclusion, our results over the years show the complex responses of a physiologic system such as the nose. The data presented support the notion of inflammation induced by hyperosmolar stimuli and also support the concept that patients with limited ability to condition inspired air are those who are subject to diseases of the airways, namely, allergic rhinitis and asthma. The interaction between allergic inflammation and water transport remains to be elucidated further.

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Does rhinitis lead to asthma?

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SUMMARY

Rhinitis and asthma are commonly linked even if the precise pathological mechanisms explaining the relationship are not fully understood. Although there is increasing evidence that rhinitis may influence the development of asthma, there remain many gaps in our understanding of the processes involved.

The complexity of this relationship is mainly due to the multiple interactions between genetic background, environmental factors and the specific host reaction. Epidemiological surveys have highlighted significant clinical associations and identified some factors that favour the progression from rhinitis to asthma. Basic research has demonstrated numerous similarities in inflammatory and immunological mechanisms.

Key words: rhinitis, asthma, epidemiology, pathogenesis, pharmacotherapy

INTRODUCTION

Since the publication in 2001 of the Allergic Rhinitis and its Impact on Asthma (ARIA) report [(1)], the relationship between rhinitis and asthma has been the scope of recent epidemiological surveys, basic research studies, and clinical trials. Results of a recent survey suggested that comorbid asthma and allergic rhinitis substantially impact patient well-being and that the worsening of allergic rhinitis symptoms in patients with asthma can be associated with worsening asthma symptoms [(2)].

Evidence shows that rhinitis and asthma are intimately linked but that some major gaps in our knowledge remain.

I. DOES EPIDEMIOLOGICAL EVIDENCE OF SUCH A RELATIONSHIP EXIST?

Rhinitis is a risk factor for asthma independent of allergy. Epidemiological studies have consistently shown that asthma and rhinitis often co-exist in the same patients [(1,3-7)]. In an international cross-sectional study in young adults, 74-81% of subjects with asthma reported symptoms of rhinitis. Conversely, the risk of asthma increased from 2% in subjects without rhinitis to 6.7-18.8% in subjects with allergic rhinitis [(8)].

Asthma is more frequently associated with perennial allergic rhinitis (PAR). Furthermore more severe asthma was associated with PAR compared to seasonal allergic rhinitis (SAR) [(9,10)].

Recent epidemiological studies reported the prevalence of asthma in allergic rhinitis patients as 20.4% [(11)] and 24% [(12)] compared to 3.9% and 2%, respectively, in controls. In two separate epidemiological studies [(12-14)], the prevalence of asthma in intermittent and persistent allergic rhinitis was the same. In children, allergic rhinitis is often diagnosed later than asthma [(15)]. This may be due, at least in part, because rhinitis symptoms in childhood are often ignored. Some prospective longitudinal studies actually suggest the opposite, namely that rhinitis frequently precedes the development of asthma. Data from Wright and colleagues [(16)] showed that children whose allergic rhinitis began in the first year of life had more respiratory symptoms at age six and were more likely to have a diagnosis of asthma.

Other prospective and longitudinal studies supported the view that rhinitis frequently precedes the development of asthma [(17-23)]. Because rhinitis and asthma are so strongly associated in cross-sectional surveys, and because rhinitis often precedes the development of asthma, rhinitis itself might be a risk factor for

asthma [8]. Allergic rhinitis has also been found to be associated with an increased risk of bronchial hyperresponsiveness in population-based study, even in subjects without diagnosed asthma [24]. In a study of patients with persistent allergic rhinitis, 54 % showed signs of early bronchial impairment and nasal function was firmly related to bronchial calibre and bronchial hyperreactivity (BHR) grade [25].

In Europe, 18 birth cohort studies on asthma and atopic diseases have been identified with in predominately urban/metropolitan settings [26].

II. WHICH MECHANISMS ARE SUSPECTED TO LINK RHINITIS WITH ASTHMA?

Bronchial asthma and rhinitis are both manifestations of an inflammatory process within a continuous airway system [27-29]. The upper and lower airway may be considered as a unique entity, influenced by a common and probably evolving inflammatory process [1], which may be sustained and amplified by intertwined mechanisms of several risk factors.

II.a. Could anatomical similarities between upper and lower airways help explain the link between rhinitis and asthma?

The histological features of the nasal and bronchial mucosa have several similarities. Both are characterized by a pseudostratified epithelium with columnar, ciliated cells resting on a basement membrane. Underneath the epithelium, in the submucosa, vessels, and mucous glands are present with structural cells (fibroblasts), inflammatory cells (essentially monocytic cells, lymphocytes and mast cells), and nerves. There are also striking differences. In the nose, there is a large subepithelial capillary and arterial system and venous cavernous sinusoids. This high degree of vascularisation is a key feature of the nasal

Table 1. Levels of evidence for treating rhinitis and asthma: rhinitis treatment influencing the course of asthma.

Class	Evidence	Drug	Reference	Type of study	Effect
Antihistamines	Ib	Cetirizine	Grant JA, JACI 1996 [69]	Randomized, double blind, placebo controlled	+ asthma symptoms
	Ib	Terfenadine	Rafferty P, Br J Clin Pharmacol 1990 [72]	Randomized, double blind, placebo controlled, crossover	+ asthma symptoms
			Taytard A, Br J Clin Pharmacol 1987 [73]	Randomized, double blind, placebo controlled, crossover	+ asthma symptoms
	Ib	Levocetirizine	Pasquali M, Cllin Exp Allergy 2006 [84]	Randomized, placebo controlled,	+ QOL
	Ib	Desloratadine	Reinartz SM, Allergy 2005 [80]	Randomized, placebo controlled,	+ allergic inflammation
			Berger WE, Ann Allergy Asthma Immunol 2002 [81]	Randomized, double blind, placebo controlled	+ asthma symptoms
			Baena-Cagnani Int Arch Allergy 2003 [82]	Randomized, double blind, placebo controlled	+ asthma symptoms and beta-agonist use
Cromones	Ib	Cromolyn	Welsh PW, Mayo clin Proc 1987 [111]	Randomized, placebo controlled	+ asthma symptoms
Nasal steroids	Ia	Nasal steroids	Taramarcaz, Cochrane Rev 2003 [93, 94]	Meta-analysis	+ but no significant effect on asthma symtpoms
Immunotherapy	Ib	Subcutaneous immunotherapy	Bahceciler NN, Pediatr Pulmonol 2001 [107]	Randomized, double blind, placebo controlled	+ asthma exacerbations and PEF
	IIa	Sublingual immunotherapy	Marogna M, Allergy 2004 [105]	Randomized controlled open study	+ BHR and asthma symptoms
Leukotriene modifiers	Ib	Montelukast	Baena-Cagnani Int Arch Allergy 2003 [83]	Randomized, double blind, placebo controlled	+ asthma symptoms and beta-agonist use
			Perry T, Ann Allergy Asthma Immunol 2004 [102]	Randomized, double blind, placebo controlled	+ lower and upper airway responses
Anti-IgE	Ib	Omalizumab	Vignola M, Allergy 2004 [110]	Randomized, double blind, placebo controlled	+ asthma exacerbations and QoL

Table 2. Levels of evidence for treating rhinitis and asthma: asthma treatment influencing the course of rhinitis.

Class	Evidence	Drug	Reference	Type of study	Effect
Inhaled steroids	IIa	Budesonide	Greiff L, Eur Respir J 1998 [112]	Not randomized, placebo controlled	+ nasal symptoms and inflammation

mucosa and changes in vasculature may lead to severe nasal obstruction. On the other hand, in the nose, there is no airway smooth muscle, whereas in the lower respiratory tract, smooth muscle is present from the trachea down to the bronchioles which accounts for bronchoconstriction as a cardinal feature of asthma. Although both diseases are caused by similar environmental risk factors, these structural end-organ differences may account, at least in part, for the differences in the clinical manifestations and severity of allergic rhinitis compared to bronchial asthma.

II.b. What are the physiological links between the nose and the lung?

The nose is a natural airway, and breathing through the nose rather than the mouth is essential for protection of the lower airway against contaminants in inhaled air [30]. The two major functions of the nose are to maintain normal airway function and to filter and condition inspired air, which contains potentially harmful particles, such as pollen grains or other allergens, inorganic dust particles or microbes, all of which could damage the bronchial mucosa if they reached the lungs. During its passage in the nasal cavity, particles larger than 5 - 10 μm are filtered out. Another important function of the nose is to warm and humidify incoming air.

Many patients with seasonal allergic rhinitis also have lower respiratory symptoms such as cough and wheeze, and many experience lower respiratory tract symptoms, particularly when the pollen count is high. Allergic rhinitis with associated nasal obstruction may result in pollen grains reaching the bronchial mucosa and resulting in symptoms of bronchial asthma. Although there is evidence of inflammation in the bronchial mucosa in seasonal rhinitis, remodelling of the bronchi, which characterises perennial asthma, is usually absent and bronchial symptoms subside at the end of the pollen season [1].

II.c. What can we learn from the immunological and pathophysiological relationships between rhinitis and asthma?

Allergic rhinitis and asthma are characterized by similar inflammatory processes in which mast cells, basophils and eosinophils play a defining role [31].

Imbalance between Th2 and Th1 cells, in favour of Th2 plays an important role in the regulation of IgE synthesis and cell recruitment at sites of allergic inflammation. In allergic rhinitis, many studies have demonstrated that mucosal inflammation is characterized by the tissue infiltration of T-lymphocytes (CD4+ T-cells and CD25+ T-cells) in the submucosa and epithelium [32,33]. These pathophysiological characteristics are found in both allergic rhinitis and asthma.

Synthesis of allergen-specific IgE is required for the development of allergic diseases including allergic rhinitis and allergic asthma but many individuals with allergen-specific IgE do not develop symptoms [34].

It is likely that inflammation in the nasal mucosa may contribute to worsening of bronchial asthma through several putative mechanisms [35]. The same inflammatory cells (T cells, eosinophils) and Th2-like cytokines have been found in nasal and bronchial biopsy specimens [36]. The number of eosinophils in nasal smears correlates well with abnormalities of pulmonary function tests and the level of non-specific bronchial responsiveness as measured by methacholine inhalation challenge [37]. In a study of patients with allergic rhinitis without bronchial asthma, segmental bronchial allergen provocation resulted in nasal inflammation characterised by tissue eosinophilia and upregulation of the eosinophil-specific adhesion molecule VCAM-1. Conversely, nasal allergen provocation resulted in allergic inflammation detectable in both the nasal and bronchial mucosa [38]. Several mechanisms have been proposed to explain the link between uncontrolled allergic rhinitis and the occurrence or worsening of bronchial asthma. These include: (a) the existence of a neural (naso-bronchial) reflex, (b) possible post-nasal drip of inflammatory cells and/or mediators from the nose into the lower airway, an event that is most unlikely (c) absorption of inflammatory cells and/or mediators from the nose into the systemic circulation and ultimately ending up in the bronchi, and (d) nasal obstruction resulting in a reduction in filtration, humidification, and warming of incoming air [35,39].

However, these mechanisms are unlikely singley to explain the entire pathological link between allergic rhinitis and asthma, as both diseases can be clinical manifestations of a systemic inflammatory process within the respiratory tract. In a nasal challenge study with house dust mites in adult patients with persistent allergic rhinitis, all patients (n=20) produced a similar early- and late-phase response by presenting nasal symptoms, inflammatory cell infiltration and mediator release in nasal secretions after challenge. Only three patients (3/5) with a history of asthma showed a fall in FEV1 readings (33%, 22% and 11% from the baseline) at seven hours post challenge and concomitant mild wheezing at night [40]. This study showed that nasal provocation may elicit concomitant asthmatic symptoms during the late phase reaction, especially in patients with a history of asthma.

II.d. Can genetic research solve the problem?

Atopy, the predisposition to develop IgE to common inhaled allergens, is a key underlying pathogenic mechanism in both allergic rhinitis and asthma. Atopy has a strong familial tendency, starting usually in childhood or adolescence [41]. Many candidate genes have been identified both by positional cloning and by linkage analysis [42-44]. A genome wide search has shown associations between certain phenotypes of allergic disease with markers on more than 14 pairs of chromosomes (chromosomes 1, 2, 3, 5, 6, 7, 9, 11, 12, 13, 14, 16, 17, 19). The complex mechanisms of inheritance of atopy and their relation to the development of clinical manifestations of atopy (allergic diseases), remain incompletely understood.

II.e. Could other environmental factors play a role?

The gene/environment interface is critical in the expression of

Table 3. Levels of evidence for treating rhinitis and asthma: rhinitis treatment preventing the allergy march.

Class	Evidence	Therapies	Reference	Type of study	Effect
Immunotherapy	Ib	Subcutaneous immunotherapy in children	Moller C, J Allergy Clin Immunol 2002 [123]	Randomized	Reduction in development of asthma
		Sucutaneous immunotherapy in children	Niggeman B. Allergy 2006 [108]	Randomized	Reduction in development of asthma
		Subcutaneous immunotherapy in adults	Polosa R, Allergy 2004 [121]	Randomized, placebo controlled	Prevention of natural progression to asthma
	Ia	Sublingual immunotherapy in children	Calamita Z et al. Allergy [109]	Meta-analysis	Beneficial but effect not large
	II	Nasal immunotherapy	Olivieri M, J Investig Allergol Clin Immunol 2000 [125]	Randomized, not placebo controlled	Onset of bronchial asthma
Antihistamines	Ib	Terfenadine	Ciprandi G, Allergy 1999 [71]	Randomized, double-blind, placebo controlled	Decrease respiratory symptoms and allergic inflammation
	Ib	Cetirizine	ETAC study group, Ped Allergy Immunol 1998 [116]	Randomised, double blind, placebo controlled	Reduction in development of asthma

Table 4. Levels of evidence for treating rhinitis and asthma. Combination therapies are better than single therapies.

Class	Evidence	Reference	Type of study	Effect
Becomethasone dipropionate inhaled and intranasal	IIa	Stelmach R, Chest 2005 [100]	Not randomized, double blind	No difference between nasal or combined therapy
Fluticasone/salmeterol with or without montelukast or fluticasone nasal spray	Ib	Nathan RA, Chest 2005 [127]	Randomized, not blinded, placebo controlled	No difference between nasal or combined therapy
Zafirlukast with nasal budesonide	Ib	Benitez HH, Rev Alergol Mex 2005 [128]	Randomized, double blind	Combination more effective
Intranasal and inhaled fluticasone	Ib	Dahl R, Allergy 2005 [129]	Randomized, double-blind, placebo controlled	Combination more effective
Loratadine and montelukast	IV	Currie GP, Q J Med 2005 [133]	Review	Combination more effective
Loratadine and zafirlukast	IIb	Roquet A, Am J Respir Crit Care Med 1997 [131]	Quasi experimental study	Combination more effective
Budesonide and montelukast	Ib	Price DB, Allergy 2006 [132]	Randomized, double-blind	Combination more effective

clinical manifestations of allergy and, most likely, in the influence of rhinitis on asthma. There are insufficient epidemiological data on the interaction between pollutants and rhinitis. Furthermore, no clear differentiation can be made between the allergens that provoke asthma and those inducing rhinitis. It seems that, probably based on the nose's poor filtration ability with respect to low molecular weight compounds [45,46], rhinitis is less common than asthma in occupational-type allergic reactions against these agents [47,48].

There is only scanty evidence on the mechanisms of occupational-type respiratory allergies.

Natural exposure studies and provocation challenges are usually poorly designed for demonstrating the mechanisms supporting the relationship between rhinitis and asthma.

Finally, the direct or indirect influence of pollution or early life infections on rhinitis and/or asthma remains unclear. At present there is little evidence to support the routine recommendation of physical or chemical methods to control indoor allergen levels, in particular allergens from furry pets [49]. Although interventional studies in adults have shown little benefit, the majority of the studies in children suggest that environmental control measures may be of benefit [50].

II.f. Which questions are remaining to explain the link between rhinitis and asthma?

Although rhinitis and asthma frequently coexist, there exist

patients that are affected by only one of these disorders. Until now, these patients are clinically classified independently of each other and a possible evolution from one disease to the other is usually neglected. There is much heterogeneity in the definitions of rhinitis employed by epidemiologists, physicians and researchers. Standardization of definitions and methods of classification are urgently needed.

The natural history of rhinitis and asthma, the chronology of events, the parameters that favour the development of rhinitis, asthma or both is poorly explored, making the categorization of patients highly variable between studies. No guidelines are available for the systematic evaluation and comparison of different studies of the link between rhinitis and asthma.

For effective diagnosis and management of rhinitis, even if specific IgE antibody determination is a necessary step for diagnosis [51], there is a clear need to develop new diagnostic tools for early categorization of airway allergic patients. Development and validation of novel in vitro testing methods [52] and measurements of new inflammatory parameters or markers of remodelling are needed to allow better labelling of patients with only rhinitis, only asthma and subjects with both rhinitis and asthma. Such precise classification will permit testing of novel hypotheses concerning the relationship between rhinitis and asthma.

III. TREATMENT OF RHINITIS AND ASTHMA

Finally, there is evidence suggesting that co-morbid allergic rhinitis is a marker for asthma resistant to treatment and worsened asthma outcomes [53]. It highlights the potential for improving asthma outcomes by following a combined therapeutic approach to co-morbid allergic rhinitis and asthma rather than targeting each condition separately.

Clinical trials represent an important source of information for investigating the impact of rhinitis on asthma. By targeting one organ and acting on one specific phase of the pathomechanism, some important information could be collected.

III.a. Does rhinitis treatment influence the course of asthma?

An adequate treatment of allergic rhinitis in asthmatics has been shown to improve asthma symptoms [54-57], pulmonary function tests [55] and to reduce costs [58]. The risk of emergency room treatment or hospitalisations [59], exercise-induced asthma [60] or bronchial hyperesponsiveness [61,62] were also shown to have reduced.

Furthermore, inadequately controlled allergic rhinitis in asthmatic patients can contribute towards increasing asthma exacerbations and poorer symptom control, which may increase medical resource use [63]. The treatment of allergic rhinitis reduces the number of asthma-related hospitalisations and emergency department visits [64-66].

III.a.1. Antihistamines

Oral H1-antihistamines represent the first-line treatment of allergic rhinitis and must not be considered as first-line treatment in asthma. However, some studies with antihistamines have found a modest effect on asthma symptoms [67-71]. In most of the studies evaluated, antihistamines were administrated at higher than recommended doses and whereas symptoms improved, objective measures including pulmonary function tests and/or peak flow rates were often unchanged [72-76]. In general, whereas antihistamines may reduce peak seasonal wheezing associated with associated severe rhinitis symptoms, these drugs are not recommended for the treatment of asthma [77-79] and inhaled corticosteroids and long-acting bronchodilators must be preferred.

In patients with allergic rhinitis and concomitant asthma, cetirizine relieves upper and lower respiratory tract symptoms [77]. Desloratadine therapy improved allergic rhinitis, and the early bronchial response [80], asthma symptoms and reduced the need for beta-agonists [81] whilst not altering pulmonary function in patients with concomitant seasonal allergic rhinitis and asthma [82]. In addition, desloratadine was as effective as montelukast in reducing symptoms associated with asthma [83]. Finally, treatment with levocetirizine decreased both symptoms and improved quality of life (Rhinasthma questionnaire) in patients with persistent allergic rhinitis and asthma [84]. Prolonged therapy over 6 months with levocetirizine reduced co-morbidities including asthma in patients with persistent allergic rhinitis and improved rhinitis-specific quality of life [85]. The effects of the novel agents ebastine and rupatadine have yet to be tested in bronchial asthma in double-blind, placebo-controlled studies.

III.a.2. Intranasal glucocorticosteroids

Intranasal treatment with glucocorticosteroids (GCS) has been found to moderately improve asthma in some but not all studies [86-92].

A recent review identified a trend for a beneficial effect in asthma but no firm conclusions could be drawn [93]. Importantly, a recent Cochrane Airways review concluded that since AR and asthma patients treated with intranasal GCS did not show appreciable differences compared with patients who were not treated, the combination of intranasal plus intrabronchial corticosteroids should remain the current clinical practice pending more research [94].

Nasal beclomethasone prevented a seasonal increase in bronchial responsiveness in patients with allergic rhinitis and asthma [95,96]. A number of aspects, such as the extent to which the pathophysiology of the two diseases overlap, and whether treating one will affect the other, remains to be clarified. Triamcinolone acetonide nasal spray blocked the increase in bronchial hyperreactivity to metacholine after high-load natural pollen exposure in children with seasonal allergic rhinitis [97] and markers of lower airways inflammation [98]. Treatment of allergic rhinitis with intranasal glucocorticosteroids significantly reduced the level of cys-LTs, a major marker of lower airway inflammation, in exhaled breath condensate [99].

Although it has been suggested that some patients with asth-

ma and rhinitis can be controlled by use of nasal medication [100], cross-sectional analysis of the effectiveness of nasal corticosteroids on asthma outcomes may result in considerable exaggeration of the protective effect of these medications in preventing severe asthma exacerbations [101].

III.a.3. Oral glucocorticosteroids

Oral glucocorticosteroids are highly effective in the treatment of rhinitis and asthma but their long-term use for this indication is restricted by severe side effects.

III.a.4. Leukotriene modifiers

Leukotriene modifiers were shown to be effective in controlling the symptoms of mild to moderate asthma and the symptoms of rhinitis [102], and the use of asthma and rhinitis medication is reduced [103]. However, a large number of patients from 2-3 studies was needed to show a 5% difference from placebo that is clinically of limited value.

III.a.5. Immunotherapy

Specific immunotherapy is effective for patients with perennial allergic rhinitis with asthma, and improves significantly their lung function [104]. It can halve the clinical score and reduces bronchial hyperreactivity [105-107]. Immunotherapy for 3 years with standardized allergen extracts of grass and/or birch showed long-term clinical effect and had a preventive effect on development of asthma in children with seasonal rhinoconjunctivitis [108]. Finally, a recent Cochrane analysis concluded that sublingual immunotherapy is beneficial for asthma treatment, albeit the magnitude of the effect is not very large [109].

III.a.6. Other therapies

Omalizumab, an anti-IgE medication, was shown to be effective in preventing asthma exacerbations in patients with concomitant asthma and persistent allergic rhinitis [110]. However, clinical applications are limited by the high cost of this medication and nowadays, it cannot be considered as first-line therapy.

III.b. Does asthma treatment influence the course of rhinitis?

Less is known about the effects on nasal disease from inhaled (intra-bronchial) treatment with glucocorticosteroids. A study examined the effects on nasal allergic disease of inhaled budesonide (avoiding nasal deposition of the drug) in patients with seasonal allergic rhinitis but without asthma [111]. During the birch pollen season, budesonide reduced the seasonal eosinophilia both in the circulation and in the nose and produced an attenuation of seasonal nasal symptoms. Nasal and systemic anti-eosinophil actions are produced at commonly employed dose levels of orally inhaled budesonide.

Theophylline was found to reduce nasal inflammation [112]. It was also observed that theophylline can reduce bronchial hyperresponsiveness in patients with allergic rhinitis [113]. However, no controlled data exist concerning the therapeutic effect of this drug on nasal symptoms.

A high percentage of asthmatics have coincidental rhinitis and so treatment for asthma that is also beneficial for rhinitis would be applicable to many patients with asthma. Such approaches include humanized monoclonal antibodies against IgE, drugs inhibiting eosinophilic inflammation or inhibiting allergic inflammation.

III.c. Can an adequate treatment of rhinitis prevent the allergic march?

The prevention effect of pharmacological treatment of allergic rhinitis on seasonal asthma is highly controversial [114] and more data are needed to fully appreciate this effect.

In infants with house dust mite or grass pollen sensitisation, treatment with antihistamines may exert a prophylactic effect on asthma onset. In the ETAC® (Early treatment of the atopic child) trial, a multi-country, double-blind, randomised, placebo-controlled trial, when considering the total group of children, no significant difference was visible but cetirizine halved the number of patients developing asthma in the subgroups sensitised to grass pollen or to house dust mite [115].

Children with pollen allergy were treated for allergic rhinitis in an open trial of three-year specific immunotherapy or allergy vaccination. Results indicate that allergen vaccination with grass or tree pollen may reduce the development of asthma in children with allergic rhinitis [116,117]. Treatment with allergen immunotherapy could lower the risk of the development of new asthma cases in adults with allergic rhinitis [118]. In routine clinical practice, specific immunotherapy could, in some way, slow the march of allergy [119-122]. This should also be the case for sublingual immunotherapy in children [123] or adults [124] with allergic rhinoconjunctivitis. The working mechanisms leading to this remain unclear including the characteristics of patients susceptible to be good responders.

III.d. In asthmatics with allergic rhinitis, is a combination of therapies more effective than a single therapy?

It has been shown that loratadine plus pseudo-ephedrine improved nasal and asthma symptoms, pulmonary function and quality of life in patients with seasonal allergic rhinitis and concomitant mild asthma [125].

In patients with persistent asthma treated with fluticasone proprionate/salmeterol, the addition of montelukast or fluticasone proprionate aqueous nasal spray for the treatment of seasonal allergic rhinitis resulted in no additional improvements in overall asthma control compared with fluticasone proprionate/salmeterol alone [126]. The association of a nasal steroid (budesonide) with a leukotriene modifier (zafirlukast) was more effective for controlling nasal symptoms and especially bronchial symptoms than the association of a nasal steroid (budesonide) with antihistamines (loratadine) with pseudoephedrine [127]. In patients with pollen-induced rhinitis and asthma, the combination of intranasal and inhaled glucocorticosteroids (fluticasone) is needed to control the seasonal increase in nasal and asthmatic symptoms [128].

Several controlled studies suggest that combination therapy with antihistamines and antileukotrienes may be as effective as corticosteroid use in patients with allergic asthma and seasonal allergic rhinitis [129,130]. There is evidence in favour of the use of anti-leukotrienes to treat asthma and also rhinitis but more data are needed to fully evaluate their full potential. Moreover, the combination of anti-leukotriene and H1-antihistamine produces a predominant inhibition of allergen-induced allergy and late-phase airway obstruction in asthmatics [131]. In asthmatics with allergic rhinitis, a combined treatment approach that included montelukast and budesonide provided significantly greater, but limited efficacy in reducing airflow obstruction when compared with doubling the dose of budesonide [132]. However, complementary cost analyses are needed before supporting such a strategy and also direct comparisons with alternative strategies such as the addition of a theophylline or a long acting inhaled bronchodilator in terms of effect size and cost.

III.e. What questions remain?

As reported above, the major studies have focused on the possible influence of rhinitis treatment on the future course and outcome of bronchial asthma. Data analysing the inverse relationship are more fragmented. Furthermore, no specific treatment is proposed for patients combining rhinitis and asthma. They are usually treated for both conditions independently with the exception of allergen immunotherapy which has been shown to be effective for both conditions and no general therapy is usually prescribed. The duration of treatment also requires further evaluation.

The possible interaction between local treatments for upper and lower airways is poorly explored and no clear guidelines are available for adapting treatment options in concomitant rhinitis and asthma. Whether or not treating rhinitis improves asthma *per se*, it is important to recognise and treat rhinitis in patients with bronchial asthma because this improves their symptoms and quality of life, whether or not the asthma improves. Finally, only limited data exists for the possible preventive role of early treatment of atopic children in the development of clinical rhinitis and asthma, whereas the early indications from long term studies of allergen immunotherapy in children with rhinitis are encouraging.

CONCLUSIONS

Epidemiological surveys have highlighted important clinical associations between rhinitis and asthma and have also identified both genetic and environmental factors that may influence disease development. Basic research has demonstrated the numerous similarities in inflammatory and remodelling pathomechanisms. New models are required to enable better differentiation of patients suffering from rhinitis and asthma. Clinical trials with current medications for rhinitis and asthma have provided new insights on the complex link between rhinitis and asthma.

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Prevalence of rhinitis among Brazilian schoolchildren: ISAAC phase 3 results*

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SUMMARY

Objective: *The International Study of Asthma and Allergies in Childhood (ISAAC) is a standardized method that allows international and regional comparisons of asthma and allergic diseases prevalence. The objective of this study was to evaluate the prevalence of rhinitis and related symptoms among 6-7 year-old children (SC) and 13-14 year-old adolescents (AD) from 20 Brazilian cities applying the ISAAC's standardized written questionnaire (WQ).*

Methods: *ISAAC's WQ was applied to 23,422 SC and 58,144 AD living in different regions of Brazil: North (N), Northeast (NE), Middle West (MW), Southeast (SE), and South (S).*

Results: *The prevalence of rhinoconjunctivitis in the last year ranged from 10.3% to 17.4% and from 8.9% to 28.5% among SC and AD, respectively. Considering SC the highest values were observed in SE region. In NE, the prevalence in countryside centres was higher than those along the coast. Among AD, the highest values were observed in N and S regions, mainly in Pará (Belém). The evaluation of populations probably with the same genetic background has shown higher prevalence among those living in urban centres (capital) in comparison to those in the countryside.*

Conclusions: *The prevalence of rhinitis and related symptoms were variable and predominate in Brazilian N and NE centres.*

Key words: children, rhinitis, rhinoconjunctivitis, prevalence, ISAAC, epidemiology

INTRODUCTION

Although rhinitis is a common disease, it is surprising that little is known about its epidemiology [(1)]. Rhinitis symptoms may occasionally occur in normal individuals, and the lack of standardized and properly validated methods for the identification of rhinitis may account for the scarcity of epidemiologic data available. In general, clinical definition of rhinitis is focused on the identification of patients whose symptoms are severe enough to require medical attention [(1,2)]. On the other hand, epidemiologic definition relies on the nature and distribution of symptoms within a population, independently from its severity [(1-3)].

The International Study of Asthma and Allergies in Childhood (ISAAC) was idealized to maximize the value of epidemiologic studies in asthma and allergic diseases, establishing a standardized method (written questionnaire, WQ) to enable international collaboration [(2,4,5)]. The ISAAC's main points are to examine variations in time trends of asthma, allergic rhinitis and atopic eczema around the world, and assess the relationship between patterns found and environmental data [(2,4,5)]. The use of a standardized WQ in ISAAC made possible to study allergic diseases among children of culturally distinct areas. This low-cost instrument has high sensitivity and specificity [(2,4,5)]. Originally written in English, the WQ was translated and validated to be applied to people of different languages, as Portuguese (Brazilian culture) [(6-8)].

Before the advent of ISAAC, little was known about the prevalence of asthma, allergic rhinitis and atopic eczema in developing countries. In Brazil, the ISAAC - Phase 1 survey, performed from 1994 to 1996, showed that the prevalence of asthma and allergic diseases among schoolchildren was not uniform throughout the country [(8-10)].

In 2002, ISAAC Phase 3 began, with the main objective of examining time trends in the prevalence of asthma and allergic diseases in centres and countries that have participated in ISAAC Phase 1 [(4,5)].

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Would the increased number of participating centres, from all regions of Brazil, give us more details about the prevalence of rhinitis and related symptoms in the country? Could these data let us know more about the real distribution of allergic rhinitis in Brazil? In this study we evaluated the prevalence of rhinitis and related symptoms in schoolchildren from 21 Brazilian centres in 20 Brazilian cities from all regions of the country.

PATIENTS AND METHODS

Centers

Twenty-one centres from 20 Brazilian cities have participated in this study. The evaluated schoolchildren were selected as standardized by the ISAAC protocol [2,4,5]. The cities, states, regions/areas, on which ISAAC Phase 3 was carried out were the following: Manaus (Amazonas, North [N]); Belém (Pará, N); Natal (Rio Grande do Norte, Northeast [NE]); Recife (Pernambuco [PE], NE); Caruaru (PE, NE); Maceió (Alagoas, NE); Aracaju (Sergipe, NE); Feira de Santana (Bahia [BA], NE); Salvador (BA, NE); Vitória da Conquista (BA, NE); Brasília (Distrito Federal, Middle-West); Belo Horizonte (Minas Gerais, Southeast [SE]); Nova Iguaçu (Rio de Janeiro, SE); São Paulo (West and South, São Paulo [SP], SE); Santo André (SP, SE); Curitiba (Paraná, South [S]); Itajaí (Santa Catarina, S); Passo Fundo (Rio Grande do Sul [RS], S), Porto Alegre (RS, S) and Santa Maria (RS, S). Some of these centres had their data approved by the ISAAC International Data Centre and were considered as ISAAC's official centres (Tables 1 and 3).

Patients

ISAAC WQ, previously translated and validated to the Brazilian culture [6-8] was applied to 23,422 children (SC, 6-7 year-old) and to 58,144 adolescents (AD, 13-14 year-old).

Subjects were selected among those who attended public and private schools located in these cities. Information regarding the number of schools and students in each area was obtained from their respective City Education Secretaries official records. After the sample definition, parents or guardians of the 6-7 year-old children and the adolescents themselves filled in the ISAAC WQ. The data obtained were transcribed to a

Table 1. Prevalence of rhinitis and related symptoms among 6-7 and 13-14 year-old Brazilian schoolchildren as determined by using the International Study of Asthma and Allergies in Childhood (ISAAC) written questionnaire in the different Brazilian centres. ISAAC Phase 3.

Centre	Latitude South	6 to 7 years old						13 to 14 years old					
		N	PNOSE EV	PNOSE 12	IEYES 12	IACTIV 12	HFEVER EV	N	PNOSE EV	PNOSE 12	IEYES 12	IACTIV 12	HFEVER EV
Belém	1.27	-	-	-	-	-	-	1,773	58.7	47.4	28.5	14.6	35.0
Manausa	3.06	3,011	27.7	19.5	10.6	13.2	13.4	3,009	35.2	23.0	12.8	14.6	21.0
Total North		**3,011**	**27.7**	**19.5**	**10.6**	**13.2**	**13.4**	**4,782**	**43.9**	**32.0**	**18.6**	**14.6**	**26.2**
Natal	5.47	855	31.5	23.3	13.3	20.8	12.7	1,020	41.4	32.0	20.0	23.8	15.3
Recife [b]	8.03							2,865	48.3	35.8	14.5	19.0	15.8
Caruaru [b]	8.17							3,026	36.9	25.5	15.4	17.5	21.6
Maceió [a]	9.39	1,990	32.0	24.7	11.3	14.3	14.7	2,745	39.4	26.4	13.8	15.3	11.1
Aracaju [a]	10.54	2,443	27.4	19.9	10.3	16.3	12.3	3,041	38.3	25.6	17.4	22.5	20.8
Feira de Santana [b]	12.16	440	40.9	35.9	15.5	24.3	26.8	1,732	41.5	33.0	17.2	25.3	18.7
Salvador [a]	12.58	998	46.9	39.8	17.4	26.0	32.3	3,020	53.6	44.2	24.4	28.2	24.2
Vitória da Conquista [b]	14.51	399	38.4	31.3	17.3	21.8	26.8	1,679	51.1	39.8	24.4	31.1	19.1
Total Northeast		**7,125**	**33.4**	**26.1**	**12.7**	**18.5**	**17.5**	**19,128**	**43.7**	**32.4**	**13.3**	**17.9**	**18.7**
Brasília [b]	15.46	-	-	-	-	-	-	3,009	42.7	29.3	15.4	21.1	20.0
Total Mid-West		**-**	**-**	**-**	**-**	**-**	**-**	**3,009**	**42.7**	**29.3**	**15.4**	**21.1**	**20.0**
Belo Horizonte [b]	19.55	-	-	-	-	-	-	3,088	38.8	26.1	14.5	18.1	24.4
Nova Iguaçu [a]	22.45	3,249	33.2	24.8	12.2	16.6	15.0	3,185	29.1	17.4	8.9	10.1	9.9
São Paulo-West [a]	23.30	3,312	34.8	28.9	15.1	19.7	21.3	3,181	39.9	30.1	19.8	20.2	18.9
São Paulo -South [a]	23.32	3,047	35.8	28.2	12.7	17.6	29.2	3,161	41.4	27.4	12.2	14.5	32.2
Santo André [a]	23.39	2,167	37.8	30.9	13.2	16.5	24.1	3,232	40.3	28.4	13.8	15.4	29.0
Total Southeast		**11,775**	**35.2**	**27.9**	**13.3**	**17.7**	**22.1**	**15,847**	**37.9**	**25.9**	**13.8**	**15.7**	**22.8**
Curitiba [b]	25.25	-	-	-	-	-	-	3,628	48.2	39.2	17.2	20.4	2.8
Itajaí [a]	26.54	1,511	25.4	19.3	13.3	14.5	16.4	2,737	31.8	22.1	12.9	14.7	19.1
Passo Fundo [b]	28.13	-	-	-	-	-	-	2,949	40.7	29.5	16.6	21.0	31.4
Porto Alegre [b]	28.15	-	-	-	-	-	-	3,007	44.5	32.1	15.9	20.0	42.1
Santa Maria [b]	28.19	-	-	-	-	-	-	3,057	30.1	20.6	9.6	15.9	20.7
Total South		**1,511**	**25.4**	**19.3**	**13.3**	**14.5**	**16.4**	**15,378**	**39.5**	**29.2**	**15.6**	**18.5**	**22.2**
Total								**58,144**	**41.0**	**29.6**	**14.6**	**17.4**	**21.4**

a = ISAAC phase 3 official centre (both age groups); b = ISAAC phase 3 official centre for 13-14ys group; N = number of schoolchildren; PNOSEEV = sneezing, runny or blocked nose ever; PNOSE12 = sneezing, runny or blocked nose in the last 12 months; IEYES12 = nose problem with itchy, watery eyes in the last 12 months; IACTIV12 = interference with daily activities; HFEVEREV = rhinitis ever.

Table 2. Odds ratios (OR) and 95% confidence interval (95%CI) of rhinitis and related symptoms in 6-7- and 13-14-year-old Brazilian schoolchildren according to the region where they live, and in the region Northeast according to the place of the centre: coastal or countryside. ISAAC Phase 3.

Question	6 to 7 years old		13 to 14 years old
	Region Northeast x Southeast OR 95% CI	Northeast Coastal x Countryside OR 95% CI	Northeast Coastal x Countryside OR 95% CI
Sneezing, runny or blocked nose ever	0.92 (0.87-098)*	0.73 (0.63-0.85)*	1.12 (1.06-1.19)*
Sneezing, runny or blocked nose in the last 12 months	0.91 (0.85-0.97)*	0.66 (0.59-0.77)*	1.71 (1.60-1.82)*
Nose problem with itchy, watery eyes in the last 12 months	0.94 (0.86-1.03)	0.71 (0.51-0.87)*	0.97 (0.90-1.06)
Interference with daily activities	1.05 (0.98-1.14)	0.72 (0.61-0.86)*	0.92 (0.85-0.98)*
Rhinitis ever	0.75 (0.69-0.81)*	0.53 (0.45-0.63)*	0.86 (0.80-0.93)*

database (Epi-Info) supplied by ISAAC's coordinators. The frequency of affirmative answers to each question was analyzed according to the age group. The Brazilian regions in which there were at least two centres were compared after they had been grouped. In NE we compared the prevalence of rhinitis and related symptoms according to the place where SC and AD lived either in coastal areas (Natal, Recife, Maceió, Aracaju, Salvador) or the countryside (Caruaru, Feira de Santana, Vitória da Conquista).

In the states of Pernambuco and Rio Grande do Sul, where the population apparently shares the same genetic background, we compared the prevalence of rhinitis and related symptoms according to the area were AD lived: capital or countryside.

Statistics

These data were analyzed by the Chi-square test and expressed as Odds Ratio (OR) with 95% confidence intervals (95% CI). We also calculated the OR and 95%CI for nasal problem associated to itchy and watery eyes in the last 12 months (allergic rhinoconjunctivitis) and daily activities limited by nasal symptoms (severe rhinitis) for each centre relative to a reference centre: Aracaju (NE, Sergipe) for SC, and Nova Iguaçu (SE, Rio de Janeiro) for AD. The association between prevalence of rhinitis and related symptoms and latitude was evaluated by the Spearman correlation index. The study was approved by all Local Ethical Committees. In all tests the level of rejection of the null hypothesis was 5%.

RESULTS

Considering both age groups the return of filled ISAAC WQ was in media 73%, varying from 62% (Aracaju) to 98% (São Paulo South).

Among SC, the prevalence of nasal symptoms without a cold sometime in their life ranged from 25.4% to 46.9%; nasal prob-

Table 3. Odds ratios (OR) and 95% Confidence intervals (95%CI) for rhinoconjunctivitis and severe rhinitis in each Brazilian centre by comparison to a reference centre (Aracaju, 6-7-year-old group; Nova Iguaçu, 13-14-year-old group).

Centre	6 - 7 years old			13 - 14 years old		
	N	Rhinoconjunctivitis OR (95% CI)	Severe rhinitis OR (95% CI)	N	Rhinoconjunctivitis OR (95% CI)	Severe rhinitis OR (95% CI)
Belém	-	-	-	1,773	4.08 (3.48-4.80)*	1.52 (1.28-1.81)*
Manaus [a]	3,011	1.03 (0.87-1.23)	0.78 (0.67-0.90)*	3,009	1.49 (1.27-1.76)*	1.52 (1.30-1.77)*
Natal	8,55	1.34 (1.06-1.69)*	1.35 (1.11-1.64)*	1,020	2.55 (2.10-3.11)*	2.78 (2.31-3.34)*
Recife [b]	-	-	-	2,865	1.73 (1.47-2.03)*	2.08 (1.80-2.42)*
Caruaru [b]	-	-	-	3,026	1.86 (1.59-2.18)*	1.89 (1.63-2.19)*
Maceió [a]	1,990	1.11 (0.92-1.34)	0.85 (0.72-1.01)	2,745	1.64 (1.39-1.93)*	1.60 (1.37-1.87)*
Aracaju [a]	2,443	1.00	1.00	3,041	2.16 (1.85-2.51)*	2.58 (2.32-2.97)*
Feira de Santana [b]	440	1.59 (1.19-2.12)*	1.65 (1.29-2.10)*	1,732	2.12 (1.78-2.53)*	3.01 (2.57-3.53)*
Salvador [a]	998	1.83 (1.49-2.26)*	1.81 (1.51-2.16)*	3,020	3.29 (2.84-3.82)*	3.50 (3.04-4.03)*
Vitória da Conquista [b]	399	1.82 (1.36-2.43)*	1.43 (1.10-1.85)*	1,679	3.30 (2.80-3.90)*	4.02 (3.45-4.70)*
Brasília [b]	-	-	-	3,009	1.86 (1.59-2.17)*	2.38 (2.06-2.75)*
Belo Horizonte [b]	-	-	-	3,088	1.73 (1.48-2.02)*	1.98 (1.71-2.29)*
Nova Iguaçu [a]	3,249	1.21 (1.02-1.43)*	1.02 (0.89-1.18)	3,185	1.00	1.00
São Paulo-Oeste [a]	3,312	1.55 (1.32-1.82)*	1.25 (1.09-1.44)*	3,181	2.53 (2.18-2.94)*	2.26 (1.95-2.61)*
São Paulo-Sul [a]	3,047	1.26 (1.07-1.49)*	1.09 (0.95-1.26)	3,161	1.42 (1.21-1.67)*	1.50 (1.29-1.75)*
Santo André [a]	2,167	1.32 (1.10-1.58)*	1.01 (0.86-1.18)	3,232	1.64 (1.40-1.91)*	1.62 (1.39-1.88)*
Curitiba [b]	-	-	-	3,628	2.13 (1.83-2.47)*	2.28 (1.98-2.63)*
Itajaí [a]	1,511	1.00 (0.82-1.24)	0.87 (0.73-1.04)	2,737	1.52 (1.29-1.79)*	1.54 (1.31-1.80)*
Passo Fundo [b]	-	-	-	2,949	2.04 (1.75-2.38)*	2.36 (2.04-2.73)*
Porto Alegre [b]	-	-	-	3,007	1.94 (1.66-2.26)*	2.23 (1.92-2.57)*
Santa Maria [b]	-	-	-	3,057	1.08 (0.92-1.29)	1.68 (1.44-1.95)*

a = ISAAC phase 3 official centre (both age groups); b = ISAAC phase 3 official centre for 13-14ys group;

Table 4. Prevalence of rhinitis and related symptoms among 13-14-year-old Brazilian adolescents according to the region of Brazil where they live.

Centre	North (N; 4,782)	Northeast (NE; 19,128)	Southeast (SE;15,847)	South (S; 15,378)	Chisquare/ Fischer
Sneezing, runny or blocked nose ever	43.9	43.7	37.9	39.5	N,NE>S>SE
Sneezing, runny or blocked nose in the last 12 months	32.0	32.4	25.9	29.2	N,NE>S>SE
Nose problem with itchy, watery eyes in the last 12 months	18.6	13.3	13.8	15.6	N>S>SE,N
Interference with daily activities	14.6	17.9	15.7	18.5	S,NE>SE,N
Rhinitis ever	26.2	18.7	22.8	22.2	N>SE,S>NE

lems in the last 12 months (current rhinitis) from 19.3% to 39.8%; allergic rhinoconjunctivitis (nose problem with itchy, watery eyes in the last 12 months) from 10.3% to 17.4%; severe rhinitis (nose problem interfering with daily activities) from 13.2% to 26.0%, and physician-diagnosed rhinitis (rhinitis ever) from 12.3% to 32.2% (Table 1).

According to the Brazilian region, data were grouped and compared: NE and SE. In SE we observed significant higher prevalence values, except for allergic rhinoconjunctivitis and severe rhinitis (Table 2). The comparison of NE centres, showed values significantly lower between those in the coast (Table 2). SC living in centres from Bahia (Feira de Santana, Salvador and Vitória da Conquista) had the highest risk of developing allergic rhinoconjunctivitis and severe rhinitis (Table 3).
Among AD, the prevalence of nasal symptoms without a cold some time in their life ranged from 29.1% to 58.7%; current rhinitis from 17.4% to 47.4%; allergic rhinoconjunctivitis from 8.9% to 28.5%; severe rhinitis from 10.1% to 31.1%, and physician-diagnosed rhinitis from 2.8% to 42.1% (Table 1, Figure 1).
The analysis of grouped data according to Brazilian regions showed values significantly higher in the N, except for severe rhinitis (Table 4). In centres from the NE region there was a higher prevalence of current rhinitis compared to those in the coast (Table 2).

The analysis of people probably with the same genetic background and inhabitanting the same area (Pernambuco [NE], and Rio Grande do Sul [S]) showed that in NE the countryside centre (Caruaru) had lower values than those observed in the capital (Recife) (Table 5). On the other hand, in S there were lower prevalences of rhinitis and related symptoms in the countryside centres (Passo Fundo and Santa Maria) when compared to the capital (Porto Alegre), except for severe rhinitis (Porto Alegre: 20.0% and Passo Fundo: 21.0%) (Table 5).
AD from Bahia's centres, Natal and São Paulo-West had the highest risk of developing allergic rhinoconjunctivitis and severe rhinitis (Table 3).
There was no significant association between Brazilian centres' latitude and prevalence of rhinitis and related symptoms (data not shown).

DISCUSSION

The ISAAC Phase 1, which was concluded in 1996 and showed a wide range of results [4,11]. The prevalence of current rhinitis has ranged from 1.5% to 41.8% among SC, and from 3.2% to 66.6% among AD; the prevalence of allergic rhinoconjunctivitis has ranged from 0.8% to 14.9% among SC, and from 1.4% to 39.7% among AD; and the prevalence of severe rhinitis has ranged from 0.5% to 28.1% among SC and from 2.2% to 57.4% among AD [11].

In Brazil, ISAAC Phase 1 has enrolled schoolchildren from seven centres of seven cities all over the country and the prevalence observed were in the middle range of ISAAC global data (10). There was a wide variation according to age. In São Paulo (SE) we observed the highest prevalence of current rhinitis, allergic rhinoconjunctivitis and severe rhinitis among SC in comparison to the other centres. Among AD the highest prevalence of current rhinitis and allergic rhinoconjunctivitis were observed in Salvador (NE) although the severity of rhinitis was higher in Porto Alegre (S). In Brazil "hay fever" is described only in S region, maybe because it has a temperate climate and seasons were better defined in comparison to other regions of the country [10]. In that study, the combination

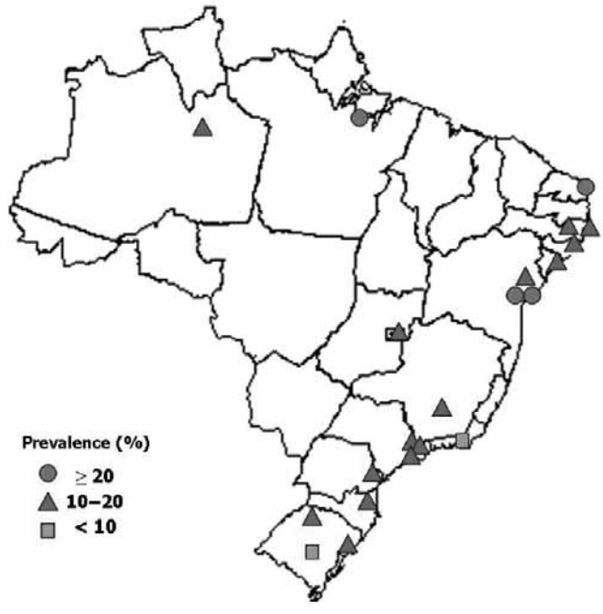

Figure 1. Prevalence of rhinoconjunctivitis among Brazilian adolescents (13-14 yrs): ISAAC phase III.

Table 5. Comparison of the prevalence (%) of rhinitis and related symptoms in 13-14-year-old Brazilian schoolchildren with the same genetic background and living in capital or countryside areas in Northeast and South region. ISAAC Phase 3.

Question	Northeast		OR	South			OR	OR
	Recife N=2,865	Caruaru N=3,026	95% CI	Passo Fundo (a) N=2,949	Porto Alegre (b) N=3,007	Santa Maria (c) N=3,057	95% CI a x c	95% CI b x c
Sneezing, runny or blocked nose ever	48.3	36.9	1.60 (1.44-1.78)*	40.7	44.5	30.1	1.60 (1.43-1.78)*	1.84 (1.68-2.07)*
Sneezing, runny or blocked nose in the last 12 months	35.8	25.5	1.64 (1.46-1.83)*	29.5	32.1	15.9	1.62 (1.44-1.82)*	1.83 (1.63-2.05)*
Nose problem with itchy, watery eyes in the last 12 months	14.5	15.4	0.93 (0.80-1.07)	16.6	20.6	9.6	1.88 (1.61-2.19)*	1.77 (1.51-2.06)*
Interference with daily activities	19.0	17.5	1.11 (0.96-1.27)	21.0	20.0	15.9	1.41 (1.23-1.60)*	1.33 (1.16-1.52)*
Rhinitis ever	15.8	21.6	0.68 (0.60-0.78)*	31.4	42.1	20.7	1.76 (1.57-1.98)*	2.79 (2.49-3.13)*

of great data variability and limited number of centres enrolled did not allow us to draw strong conclusions.

In the present study there was a 3-fold increase in the number of participating centres. Considering the SC group, as observed in ISAAC Phase 1, the prevalence of current rhinitis, allergic rhinoconjunctivitis, and severe rhinitis were in the middle range. Highest values were observed in Bahia (Feira de Santana, Salvador and Vitória da Conquista [NE]) (Table1). However, comparing regions NE and SE there were no significant differences between them except for current rhinitis that was higher in NE (Table 2).

It has been demonstrated that air pollution is one of the main causes and/or triggers of allergic respiratory diseases [12]. In SE region there are cities with the largest people agglomeration and highest air pollution indexes of Brazil (São Paulo and Santo André). In these cities prevalence rates for rhinitis and related symptoms were intermediary (Table 1). On the other hand, analyzing the NE centres more carefully, we realize that although those on the coast were state capitals with a high population density, they show lower prevalence of rhinitis and related-symptoms in comparison to those in the countryside.

Brazil is a country with continental dimensions, whose total area of 8.5 million square kilometers is cut in N by the Equator and in SE by the Tropic of Capricorn [13]. Its climate varies according to the area. In N, NE and MW areas, the climate is tropical (high temperature) with dry and lingering summers and rainy winters. In the SE and S areas, the climate is temperate, whereas seasons are better defined in S [13]. In a recent paper, Weiland et al. have studied the relation between heat and humidity and the prevalence of asthma and allergic rhinitis in children aged 6 to 7 year-old evaluating worldwide data from 146 centres of the ISAAC Phase 1 [14]. Similar to us, they did not find a positive association between prevalence of allergic rhinoconjunctivitis and latitude. However, they observed a negative association between mean annual outdoor temperature with asthma, allergic rhinoconjunctivitis and atopic eczema [14].

Evaluating the data from AD group, we observed higher prevalence of rhinitis and related-symptoms when compared to SC group. The range of prevalence of current rhinitis, allergic rhinoconjunctivitis and severe rhinitis were in the same range that was obtained in ISAAC Phase 1 [10].

The comparative analysis of Brazilian regions showed higher values of current rhinitis in N and NE regions. In opposite to those observed among SC, the rates were higher in NE coastal centres. A positive association has been reported between dampness/mold indoor exposure and the risk of developing rhinoconjunctivitis [15,16]. In N and NE centres the mean outdoors daily temperatures are higher and in Manaus, Belém and those centres along the coast the humidity is also higher. In this study we have documented high risk of current rhinitis and severe rhinitis in Belém and in centres of Bahia, similarly to that observed in SC group (Table 3). May the higher indoor dampness exposure, commonly present in these cities, be the explanation for our results?

Another point to concern is the relationship between socioeconomic status (SES) and the prevalence of rhinitis and related-symptoms. In this study, Caruaru, Feira de Santana and Vitória da Conquista - centres with the lowest SES [17] (data not shown) - are between those with higher prevalence of rhinitis and related symptoms. This fact is in contrast with the Hygiene Hypothesis [18], and was also observed previously by Mercer et al. [19].

The Hygiene Hypothesis has been postulating that early exposure to infectious agents and/or endotoxins, and to live in an environment with great number of children are some factors related to the protection against development of asthma and allergic diseases [20]. Viral infections and parasite infection were associated with low prevalence of sensitization [21,22]. In a recent study we have not observed an inverse relationship between the prevalence trend of allergic rhinoconjunctivitis and the decreasing incidence of tuberculosis and measles [23]. Likewise, Nascimento Silva et al. did not find significant differences in the prevalence of current asthma in children with and without ascariasis infestation [24].

Brazil was colonized by the Portuguese, and afterwards was invaded by people from other countries (Dutch and French), had African slaves and in the last century, received immigrants from several places of the world with consequent high degree of miscegenation. Because of this, Brazilian people have a great genetic diversity, which hampers any genetic study. The evaluation of people with apparently the same genetic background and living in different places in the same state confirmed data previously reported: lower values in centres located outside of the great centres [25].

Comparing data from ISAAC Phase 1 and 3 we observed that the prevalence of rhinitis in Brazil remained stable with a non-homogenous trend of prevalence in the cities participating [26]. Although ISAAC Phase 3 data collection has been concluded around the world, final results are unknown yet. As we had observed at the end of ISAAC Phase 1, the data obtained in this study reinforce the great variability of the prevalence of rhinitis and related-symptoms around the country. More deepened studies evaluating etiology, genetics and environmental risk factors are necessary in order to allow us to conclude more properly about rhinitis and related-symptoms among Brazilian children.

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BDNF and DPP-IV in polyps and middle turbinates epithelial cells*

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SUMMARY

Hypothesis: *Neuropeptides released from sensory nerves may contribute to airway inflammation, particularly if their metabolism is impaired through defective inactivation and/or increased production. In the airways, neuropeptides are subjected to degradation by enzymes such as dipeptidyl peptidase (DPP-IV), and are upregulated by neurotrophins such as brain derived neurotrophic factor (BDNF). We therefore assessed in primary human nasal epithelial cells the expression of DPP-IV and BDNF under basal and inflammatory conditions.*

Methods: *Human epithelial cells were isolated from nasal polyps and middle turbinates, and grown on collagen-coated polycarbonate filters with an air liquid-interface. After three weeks of culture, constitutive cellular expression of DPP-IV and BDNF was assessed by measuring its activity and by ELISA, respectively. To mimick in vivo inflammatory conditions, cells were exposed apically and basolaterally to 50 ng/ml of TNFα, IL-1β, and IFN-γ for two days. DPP-IV activity and BDNF protein expression were measured in cell lysates and in the apical and basolateral media.*

Results: *Constitutive DPP-IV activity was similar in polyps and turbinates cells. In contrast, polyps epithelial cells expressed higher amounts of BDNF compared to turbinates derived cells. On the other hand, TNFα, IL-1β, and IFN-γ did not affect DPP-IV activity but significantly increased the cellular expression and the basolateral secretion of BDNF.*

Conclusions: *Our data show for the first time that primary human airway epithelial cells produced DPP-IV and BDNF under basal conditions. Furthermore, the production and secretion of BDNF were markedly increased in response to pro-inflammatory cytokines, confirming the involvement of BDNF in airway inflammation.*

Key words: allergic airway diseases, dipeptidyl-peptidase IV, neuropeptides, neurotrophins

INTRODUCTION

The concept of neurogenic inflammation events in the airways and their potential importance for airway diseases such as allergic rhinitis and asthma have been proposed [1,2]. Inflammatory mediators may modulate cholinergic and sensory nerves in the airways through the activation of receptors on nerve terminals, and sensory nerves in turn may amplify inflammation in the airways through the release of neuropeptides such as substance P (SP) and neurokinin A (NKA). SP and NKA are potent vasodilators and bronchoconstrictors, and stimulate mucin secretion from human submucosal glands in vitro. These neuropeptides are degraded and inactivated by peptidases namely neutral endopeptidase (NEP) and dipeptidyl peptidase (DPP-IV) located at the surface of airway epithelial cells, airway smooth muscle cells, submucosal gland cells as well as fibroblasts [3]. Thus reduced activity of these peptidases leads to exaggeration of the inflammatory response evoked by peptides released from peripheral endings of sensory nerves [4].

A further family of biologically active peptides which may interact with sensory neurons and thereby propagating airway neurogenic inflammation are the neurotrophins [5]. The most prominent members of the neurotrophin family are nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3 and NT-4/5. To date, most studies concerned NGF. Subjects with allergic rhinitis have decreased NGF mRNA in nasal scrapings and increased NGF protein in nasal lavage fluids [6]. Asthmatic patients exhibit significantly enhanced NGF levels in the bronchoalveolar lavage fluid and intense NGF-immunoreactivity in bronchial epitheli-

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um, smooth muscle cells and infiltrating inflammatory cells in the submucosa [7]. The biological effects of neurotrophins are mediated through signalling by different neurotrophin receptors, which are expressed not only on nerve cells but also on immune cells including monocytes, mast cells, B cells and T cells. Thus, NGF increases expression of SP in sensory neurons, induces mast cell degranulation, cytokine synthesis and regulates antibody production. Additionally, NGF is a chemoattractant and activation factor for eosinophils [8].

Recent studies have reported an inverse relationship between DPP-IV enzymatic activity and inflammation in the nasal mucosa [4] and a positive correlation between SP content in the plasma and sputum and airway inflammation [2]. These observations led us to evaluate in this study the expression of DPP-IV, a peptidase known to be involved in SP degradation, and BDNF, a neurotrophin involved in SP synthesis, in primary human nasal epithelial cells derived from middle turbinates and polyps. Specifically, we asked whether: 1) nasal epithelial cells constitutively express DPP-IV and BDNF, if so 2) the expression of DPP-IV and BDNF is different between cells derived from turbinates and polyps, and 3) DPP-IV and BDNF expression is affected by proinflammatory cytokines.

MATERIALS AND METHODS

Cell culture

Human airway epithelial cells were obtained from different patients after surgical polypectomies and partial middle turbinectomies. Patients gave informed consent and the protocol was approved by the institution's ethical committee. Cells were isolated by pronase (Roche, Mannheim, Germany) digestion as described [9]. Freshly isolated cells were seeded at a density of 5x105 cells/cm^2 onto 0.6-cm^2 collagen-coated Millicell polycarbonate filters (Millipore, Molsheim, France). Twenty hours after plating, the mucosal media was removed and the cells are allowed to grow at the air-liquid interface, which will allow the cells to develop a morphological and functional phenotype that closely resembles in vivo airway epithelium. The culture media consists of a 1:1 mix of DMEM:F12 (Invitrogen, Life Technologies, Basel, Switzerland), 2% Ultroser G (Pall-Biosepra, Cergy-Saint-Christophe, France), 100 U/ml penicillin and 100 μ-g/ml streptomycin. After 15-20 days, the epithelial monolayers developed a transepithelial resistance of >500 ohm.cm^2.

Experimental conditions

Constitutive expression: The basal expression of DPP-IV and BDNF was measured in cells after three weeks of culture, at which time the cells were fully differentiated to polarized respiratory epithelia that displayed histologic and biochemical characteristics similar to those observed *in vivo*.

Cytokine treatment: Polarized cells were treated apically and basolaterally with 50 ng/ml of TNFα·, IL-1β, and IFN-γ (Sigma, Sigma-Aldrich Chemie, Buchs, Switzerland) for two days. After the treatment, the apical and basolateral media were removed, the cells were washed with PBS and scraped in 100 mM Tris pH 8,0 containing 2% Triton-X100 and sonicated. An aliquot was kept for protein assay and the rest was used for measurement of DPP-IV activity and BDNF protein.

Measurement of DPP-IV activity

DPP-IV activity was measured as previously described [4]. The fluorogenic substrate used was the Gly-Pro-AMC (Bachem, Budendorf, Switzerland). Gly-Pro-AMC (5 mM) were incubated for 3h at 37°C with varying volumes of cell extract in Tris–HCl buffer, pH 8,0. AMC production was monitored by fluorescence measurement (λ_{ex}=370 nm, λ_{em}=460 nm). Fluorescence intensity was related to an AMC (Bachem, Budendorf, Switzerland) standard curve, and results were normalized to protein content.

Quantification of BDNF by enzyme-linked immunoabsorbent assay (ELISA)

To quantify BDNF protein levels in cell lysates and media, commercially available ELISA kit was used according to the manufacturer's instructions (Promega, Madison WI, USA). Plates were read in a microplate reader at 405 nm. Prior to analysis, the media were concentrated using the Amicon Centricon and Microcon centrifugal filter devices (Millipore, Billerica, MA, USA) with a molecular weight cut off of 3000 Da, according to the protocol of the manufacturer.

Statistical analysis

The significance of the difference between groups ($p < 0.05$) was determined with the nonparametric Kruskal-Wallis test and the Wilcoxon signed rank test for paired samples using the Statview program for Windows (Version 5.0.1, SAS Institute Inc.). Results are given as means ± SEM.

RESULTS

DPP-IV activity and BDNF protein expression in polyps and turbinates epithelial cells

DPP-IV expression was assessed by measuring its activity and BDNF expression was measured by ELISA in cell lysates. Epithelial cells obtained from either turbinates or polyps expressed significant DPP-IV activity and BDNF protein amount. A trend toward a higher level of DPP-IV activity was observed in polyps epithelial cells compared to turbinates cells, however this difference did not reach statistical significance (Figure 1A). Polyps epithelial cells also expressed a larger amount of BDNF protein than turbinates epithelial cells, and this difference was statistically significant (Figure 1B).

Effects of pro-inflammatory cytokines on DPP-IV and BDNF

Next we tested the effects of the pro-inflammatory cytokines TNFα, IL-1β, and IFN-γ (cytomix) on DPP-IV activity and BDNF expression in nasal epithelial cells. DPP-IV activity and BDNF protein amounts were measured in both cell lysates and in the apical and basolateral media. The results are shown in Figures 2 and 3. In untreated cells, DPP-IV and BDNF were essentially released in the apical and not in the basolateral

compartment. Cytomix affected neither cellular DPP-IV activity (Figure 2A) nor the apical release of DPP-IV (Figure 2B). In contrast, cytomix significantly increased BDNF expression in cells (Figure 3A) and the secretion of BDNF into the basolateral compartment (Figure 3B).

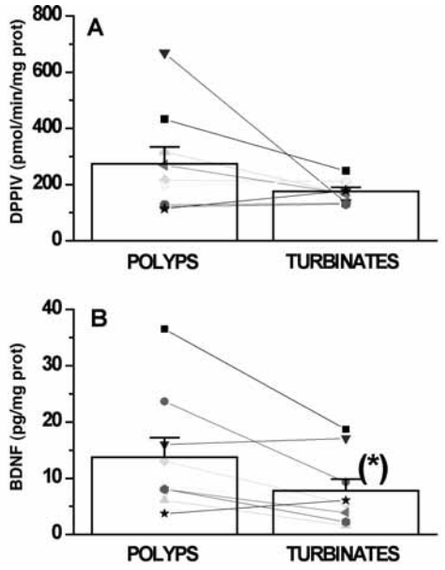

Figure 1. Basal expression of DPP-IV (A) and BDNF (B) in polyps and turbinates derived epithelial cells. Each symbol represents a patient, of which a polyp and turbinate biopsy were used to generate epithelial cell cultures. Means ± SEM (n=8) are also given, (*) $p<0.05$.

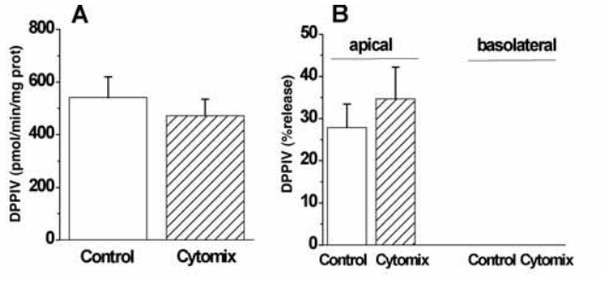

Figure 2. Effects of TNFα+IL-1β+IFNγ (cytomix) on DPP-IV activity in cell lysates (A) and in the apical and basolateral compartments (B). Results are shown as means ± SEM (n=6). % release = (DPP-IV activity in medium / total DPP-IV activity in cells + media) *100.

DISCUSSION

The results presented in this study demonstrate for the first time that 1) primary human nasal epithelial cells in culture produced DPP-IV and BDNF, 2) the production of BDNF and not of DPP-IV was higher in polyps cells than in turbinates cells, and 3) the production and basolateral secretion of BDNF and not of DPP-IV increased in response to the pro-inflammatory cytokines TNFα·, IL-1β, and INFγ. These results clearly suggest that BDNF may be involved in airway inflammation and hyper-reactivity, either through increasing production of

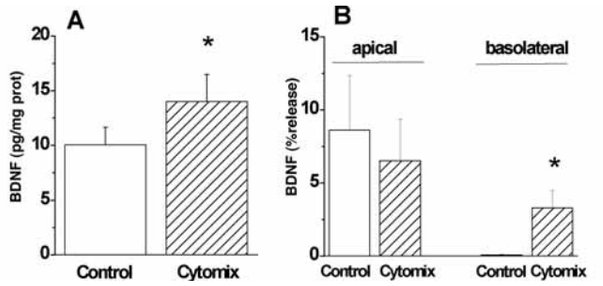

Figure 3. Effects of TNFα+IL-1β+IFNγ (cytomix) on BDNF protein levels in cell lysates (A) and in the apical and basolateral compartments (B). (*) $p<0.05$. Results are shown as means ± SEM (n=4-9). % release = (BDNF in medium / total BDNF in cells + media) *100.

neuropeptides by sensory neurons or by affecting immune cell function or both.

Neurogenic inflammation through the release of neuropeptides such as substance P, neurokinins A/B and C from nerve terminals has been suggested to have an important role in the development and progression of chronic inflammatory airway diseases. Neuropeptides are potent bronchoconstrictors and vasoactive agents. They have also potent effects on airway secretions and on inflammatory and immune cells. The bioactivity and thus the physiological effects of neuropeptides is terminated by their degradation by specific peptidases namely NEP and DPP-IV located on the membrane of a number of cell types within the lung [3]. Here we show for the first time that primary human nasal epithelial cells produced DPP-IV constitutively. This is in line with the immunostaining of human nasal mucosa where DPP-IV immunoreactivity was found in some epithelial cells [4]. Surprisingly, the proinflammatory cytokines TNFα, IL-1β, and IFNγ had no effect on DPP-IV production and secretion in our cells. Consistent with previous findings on MDCK and Caco-2 cells [10], DPP-IV was apically secreted in nasal epithelial cells. The presence of glycans on the DPP-IV ectodomain has been shown to be responsible for this apical targeting. Further studies are needed to assess the physiological significance of the apical localization of DPP-IV in nasal epithelial cells.

In situ expression of the different neurotrophins in the normal human lung was recently reported. Neurotrophins are constitutively and differentially expressed by the resident cells of human lung including bronchial mucosal epithelial cells, bronchial smooth muscle cells, bronchial gland cells, pulmonary macrophages as well as intrapulmonary arteries [11]. To date, almost all invitro studies focused on NGF, which production was demonstrated in human lung interstitial cells namely fibroblasts [12] and bronchial smooth muscle cells [13]. So far, data concerning neurotrophin expression and secretion by primary polarized human airway epithelial cells are missing. At present, the expression of NGF was reported only in the human lung epithelial A549 cell line [14,15]. Whereas A549 cells are representative of airway epithelial cells in some properties,

they are nevertheless tumour cells and it is well known that tumours have a modified expression pattern of growth and survival factors. To our knowledge, our present study demonstrates for the first time that primary polarized human nasal epithelial cells constitutively produced BDNF. Interestingly, a higher level of BDNF was found in polyps cells compared with turbinates cells. Furthermore, proinflammatory cytokines increased the intracellular protein level of BDNF and the basolateral but not the apical release of BDNF. This is of special interest since only neurotrophin secretion towards the basement membrane is of biological importance for signalling within the tissue. We can reasonably assume that BDNF might function like NGF which has been demonstrated to contribute to the inflammation and hyper-responsiveness associated with allergic diseases via effects on inflammatory cells. NGF promotes the infiltration and survival of eosinophils, suppresses apoptosis while inducing proliferation of mast cells [8], and increases tachykinin expression in airway sensory neurons [16]. It is noteworthy that, like DPP-IV, constitutive BDNF is apically secreted in primary nasal epithelial cells. In this respect, contradictory data has been reported. Thus, BDNF has been shown to be secreted predominantly in the basolateral compartment in LA-4 murine bronchial epithelial cells [17]. In transfected MDCK cells, BDNF was released either apically (18) or basolaterally [19].

In conclusion, this is the first study demonstrating DPP-IV and BDNF production by primary polarized human nasal epithelial cells. Furthermore, our study shows that the proinflammatory cytokines TNFα, IL-1β, and INFγ enhance the production and the basolateral secretion of BDNF. This finding suggests that, in vivo, the respiratory epithelia may amplify the ongoing local inflammatory process, by contributing to neurogenic inflammation through increased secretion of neurotrophins. It is now being recognized that local overproduction of neurotrophins during allergic inflammation modulate the activity of sensory neurons resulting in increased synthesis and release of neuropeptides such as substance P [20,21]. The activities of substance P include a broad range of functional responses of immune cells including lymphocytes, eosinophils, mast cells and macrophages leading to activation and differentiation of these cells [22,23]. Thus high concentrations of neurotrophins found in the serum [24] and the bronchoalveolar lavage fluid of asthmatic patients [7], as well as in the nasal fluid of patients with allergic rhinitis [25], may act as potent mediators of inflammation causing plasma extravasation and attracting inflammatory cells to the site of release. Therefore, neurotrophins may be considered as mediators of a vicious cycle of neuroimmune interactions that amplify airway inflammation and airway hyperresponsiveness in allergic diseases.

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Side-effects of injective allergen immunotherapy administered to intermittent or persistent allergic rhinitis patients*

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SUMMARY

Aim: *Evaluation of the side-effects of conventional subcutaneous allergen immunotherapy in inhalant allergy.*

Material and methods: *Retrospective analysis of early and late, local and systemic, short-term and long-term side-effects of 4723 injections given to 224 patients suffering from intermittent or persistent allergic rhinitis.*

Results: *There were 65 systemic reactions in 48 patients (21%) after 61 injections (1.29%). Most of them were late, and included dyspnoea, rhinorrhoea, fever, fatigue and urticaria. Incidence of systemic reactions did not correlate to age or sex, but was higher in grass pollen than in house dust mite allergy and during the up-dosing phase of treatment. Late intense local reactions were observed after 1.6% of injections.*

Conclusions: *Allergen immunotherapy in inhalant allergy is a safe method of treatment.*

Key words: allergen immunotherapy, side-effects, intermittent allergic rhinitis, persistent allergic rhinitis

INTRODUCTION

Allergen immunotherapy (IT) is standard therapeutic management of patients suffering from allergic diseases. This method, besides avoidance of exposure to clinically significant allergens, is the only one possibility of causal treatment of IgE-mediated diseases. IT has immunomodulation activity and additionally, it also modifies the natural history of atopic allergy. Even though specific immunotherapy is considered to be a safe method of treatment, there is a risk of side-effects during its application [(1)].

The most frequent method of IT is subcutaneous injections of increasing doses of extracts of allergens of proven clinical relevance. Allergen vaccines should be administered in optimal doses to achieve clinical efficacy. However this method of treatment poses the risk of a life-threatening anaphylactic reaction that needs immediate medical intervention. Side-effects of allergen vaccines can occur locally and systemically. Local symptoms may be early (in <30 minutes) or late (after 30 minutes, the most frequently after 6-24 hours). They manifest as excessive oedema, reddening, pain and pruritus in the place of injection. Systemic symptoms may manifest as an intensification of allergic symptoms such as rhinitis, dyspnoea, urticaria, fatigue and shock. These symptoms can occur early and late. While late symptoms, both local and systemic, are not dangerous and can be controlled through changing the dose of allergen vaccine, systemic anaphylactic reaction is life-threatening and always needs immediate medical intervention [(2-5)].

It has been generally accepted that correct indications for immunotherapy and treatment by a specialist result in the optimal therapeutic index and the highest level of safety. Even

List of abbreviations:
IT – immunotherapy
IAR – intermittent allergic rhinitis
PAR – persistent allergic rhinitis
BA – bronchial asthma
AD – atopic dermatitis
SSE(s) – systemic side effects (effects)
LSE (s) – local side effect (effects)

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though IT has been accepted as a safe method of treatment, there is a risk of side-effects. Accordingly, constant observation for side-effects of this form of immunotherapy is necessary.
The aim of our study was to estimate the frequency and severity of side-effects of allergen immunotherapy in patients with intermittent or chronic allergic rhinitis.

MATERIAL AND METHODS

Material

In total, 224 patients (129 males, [57.6%], mean age 25.07 years, range 5-46), including 127 subjects with intermittent allergic rhinitis and 97 with persistent allergic rhinitis who underwent allergen immunotherapy from 1990 to 2001, were included in this retrospective study. The patients were treated in the following institutions in Poland: Out-patient Clinic of Allergology, Centre for Pulmonary Diseases in Bystra Śląska, Out-patient Laryngological Clinic, Hospital "Stalownik" in Bielsko-Biala, Out-patient Clinic of Allergology in Zabrze, and Chair and Clinical Department of Internal Diseases, Allergology and Clinical Immunology, Silesian University School of Medicine, Zabrze.
Patients were divided into four subgroups: a group IAR - 100 subjects with intermittent allergic rhinitis, a group IAR+BA – 27 subjects with intermittent allergic rhinitis coexisting with bronchial asthma, a group PAR - 51 patients with chronic allergic rhinitis, and a group PAR+BA - 46 patients with chronic allergic rhinitis coexisting with bronchial asthma. Patients in groups IAR and IAR+BA had clinical signs of sensitization to grass, rye, tree or weed pollen. All the patients in groups PAR and PAR+BA were sensitized to house dust mites. No patient with intermittent allergic rhinitis was sensitive to perennial allergens (house dust mites) and similarly no one with persistent allergic rhinitis suffered from sensitization to seasonal allergens.
There were 8 patients with atopic dermatitis: 2 subjects in the IAR group, 4 subjects in the PAR group, and 2 subjects in the PAR+BA group.

Type of immunotherapy

The decision to use immunotherapy was made on medical history regarding the appearance or increase in disease symptoms and the positive results of skin prick tests (wheal diameter >3 mm). A solution containing phenol, glycerol and natrium chloride was used as negative control and histamine diphosphate (5 mg/ml) as a positive control.
Before applying the vaccines the following procedures took place: (i) physical examination; (ii) measurement of peak expiratory flow, (iii) assessment of local early and late reaction after a previous injection according to recommendations by EAACI; (iv) assessment of systemic reaction after a previous injection. Antihistaminic preparations were not applied as a preventive method.
Allergen immunotherapy was conducted according to generally accepted rules and manufacturers' recommendations.
The following vaccines were applied:

1. Aqueous extracts of allergens:

a. **Catalet T or D** (Company of Serums and Vaccines, Kraków, Poland; T stands for grass pollens, and D stands for tree allergens), (aqueous extract of inhalant allergens precipitated with zinc chloride and tannin, modifying formalin and aluminum hydroxide-adsorbed; standardized in PNU units).
b. **Alutard SQ** (Alk Laboratories, Denmark) (aqueous allergen extract aluminum hydroxide- adsorbed and diluted in physiologic salt solution with 0.5% phenol as conserving agent).

2. Modified allergen extracts:

a. **Allergovit** (Allergopharma, Germany) (alergoids aluminum hydroxide- adsorbed), standardized in therapeutic units,
b. **Novo–Helisen Depot** (Allergopharma, Germany) (allergenic extract with buffered salt solution, aluminum hydroxide- adsorbed, in suspension of physiologic salt solution, preserved in 0.4% phenol) standardized in therapeutic units
c. **Alavac S** (Bencard, Brentford, England; now Allergy Therapeutics Poland) (standardized allergenic extract of house dust mite, extracted and modified by pyridine, and hydroxide- adsorbed, preserved in 0.5% phenol)

Catalet T was given to 74 subjects (33%), Catalet D to 5 subjects (2%), Alutard SQ to 36 subjects (16%), Allergovit to 47 subjects (21%), Novo – Helisen Depot to 26 subjects (12%), and Alavac S to 36 subjects (16%).

Assessment of side effects

Local and systemic, early and late side-effects were evaluated according to the European Academy of Allergology and Clinical Immunology (EAACI) scale with regard to the severity of syndrome (0- IV°) [6]:
Grade 0 – without symptoms; Grade 1 – non-specific symptoms, probably independent from IgE, such as headache, discomfort, arthralgia etc.; Grade 2 – mild, systemic reactions such as weak intensification of rhinitis or asthma, which respond well to drugs (antihistaminic drugs or short-acting β2-mimetics); Grade 3 – systemic non-life-threatening reactions such as urticaria, angioedema, symptoms of asthma, which respond well to drugs; Grade 4 – anaphylactic reaction.
The final analysis was based on observations of subjects by physicians who oversaw the whole course of immunotherapy and by patients who noted symptoms down in the patient's diary and in questionnaires recommended by the EAACI [6]. All symptoms remaining in cause and effect relationship with allergen injections were accepted as side-effects. Before starting treatment informed consent from all patients or their parents in the case where patients were under age of 18 was obtained.

Table 1. Local side-effects of immunotherapy - time of onset and method of management.

Type of side-effects	Number (%) of injections which induced LSE	Time of incidence	Management
Oedema 5- 10 cm	45 (0.95)	Late (after 6- 24 hours) -45	Spontaneous resolving
Oedema >10 cm	7 (0.15)	Late (after 6-24 hours) -7	Antihistaminic drugs, cold compress
Pruritus in the place of injection	7 (0.15)	Late (after 6-24 hours) -7	Ice compress
Pain of arm	18 (0.38)	Late (after 6-24 hours) -18	Antihistaminic drugs, cold compress
Lack of side-effects	4646 (98.37)		
Together	**4723 (100)**	**Early 0 Late 77**	

Table 2. Local side-effects of immunotherapy according to type of allergic disease.

Type of allergic disease	Number (%) of injections	Type of local side-effects	Time of incidence early	late
IAR	18 (23)	edema 5-10cm – after 9 injections; edema >10 cm – after 5 injections; pain – after 4 injections;	0	18
IAR+BA	10 (13)	edema 5-10 cm – after 9 injections; pain - after 1 injection;	0	10
PAR	15 (20)	edema 5-10 cm – after 5 injections; pruritus – after 6 injections; pain – after 4 injections;	0	15
PAR+BA	34 (44)	edema 5-10 cm – after 22 injections; edema >10 cm – after 2 injections; pruritus – after 1 injection; pain – after 9 injections	0	34
Together	**77**		**0**	**77**

IAR indicates a group of patients with intermittent allergic rhinitis, IAR+BA – a group of patients with intermittent allergic rhinitis and asthma, PAR - a group of patients with persistent allergic rhinitis, PAR+BA - a group of patients with persistent allergic rhinitis and asthma.

Statistical analysis

As data were mostly qualitative, statistic analysis was based on the Chi-square test with modifications if needed (Yates (Y) correction, V- square test). All analyses were performed with a software package (The Quick STATISTICA PL.) and p-values < 0.05 were considered significant.

RESULTS

In total, 4723 injections in 224 subjects were analyzed (average 21 injections per patient), of which 3327 injections were applied during the up-dosing phase and 1396 injections during the maintenance phase.

Local side-effects

Local side-effects occurred in 33 (14%) patients after 77 (1.6%) injections. All of them were late and manifested as oedema at the site of injection and / or brachialgia which needed anti-histamines and / or compress of ice. The more severe side effects occurred in the patients suffering from chronic rhinitis with bronchial asthma. The most common local side-effects were clinically insignificant and were treated with ice compress or local anti-histamines without modification of the consecutive dose of vaccine (Tables 1 and 2).

Systemic side-effects

Among 4723 injections of allergen vaccines, which were given to patients included in analysis, 61 (1.29%) induced systemic side-effects (SSEs). Only 3.1% of them were early and 96.9% were late. SSEs occurred in 48 (21%, 26 males) patients treated with allergen vaccines. Most of these symptoms were classified as mild (1st or 2nd grade according to EAACI). The most frequent SSEs included running nose, sneezing, lacrimation and did not need treatment. Urticaria occurred in 4.6% of cases as late symptoms by 3rd grade of seriousness and regressed after using an antihistamine. Systemic reaction (4th grade according to EAACI) was not observed in any case. Dyspnoea was observed after 15 (0.32%) injections, intense rhinitis after 37 (0.79%) injections, fever after 3 (0.06%) injections, and fatigue after 6 (0.13%) injections. Anxiety due to a sleepless night occurred in 1 patient after 1 injection. There was no correlation

between sex or age of patients and the incidence of side-effects. SSEs occurred significantly more often after vaccines containing pollen allergens than those house dust mite allergens ($p < 0.00001$). Systemic symptoms were preceded by local symptoms only in two cases; there were dyspnoea and fatigue occurring at the same time as edema and redness in a site of injection. These symptoms resolved after applying antihistaminic drug. The distribution of SSEs in all groups of patients has been shown in Table 3. The most SSEs occurred in patients suffering from intermittent allergic rhinitis (Chi-square test; $p = 0.002$). There were more SSEs in the group IAR+BA in comparison with the group PAR+BA (V test; $p = 0.001$).

In patients with bronchial asthma, dyspnoea was observed after 12 injections, whereas in the group of patients with IAR only 3 injections caused dyspnoea. Dyspnoea occurred three times in patients of the group IAR+BA sensitive to grass and rye, in the up-dosing phase. Dyspnoea was mild, occurred 20 minutes after injection, with no physical signs and spontaneously resolved. In 9 (4.0%) patients of the group PAR+BA dyspnoea with wheezing was observed in the up-dosing phase. In all cases the reactions were late in their nature and regressed after inhalations of short-acting β2-mimetics. No disturbances of ventilation were noticed during next visits and at home therefore IT was continued.

SSEs occurred more often in the up-dosing phase than in maintenance phase [53 SSEs, (86.8% of all SSEs) during the up-dosing phase and 8 SSEs (13.2%) during the maintenance phase ($p < 0.0001$).] The occurrence of SSEs was 1.12% (53 of 3327 all applied injections) in the up-dosing phase of treatment, and 0.17% (8 of 1396 all applied injections) in the maintenance phase (p=0.005, Chi-square test). The occurrence of SSEs in individual years of immunotherapy did not differ significantly (data not shown).

Table 3. Distribution of systemic side-effects of immunotherapy according to type of allergic disease.

Allergic disease	Number of patients	Number (%) of patients with SSEs	Number of injections administered	Number of injections inducing SSEs
IAR	100	24 (24%)	1458	30
IAR+BA	27	9 (33%)	329	12
PAR	51	3 (6%)	1494	2
PAR+BA	46	12 (26%)	1442	17
Total	**224**	**48 (100%)**	**4723**	**61**

SSE indicates systemic side effects, IAR – a group of patients with intermittent allergic rhinitis, IAR+BA – a group of patients with intermittent allergic rhinitis and asthma, PAR - a group of patients with persistent allergic rhinitis, PAR+BA - a group of patients with persistent allergic rhinitis and asthma.

DISCUSSION

In this paper safety of injective specific immunotherapy of inhalant allergy has been proved. Side-effects relevant to administration of allergen vaccines occurred rarely and were mostly mild. Observed side-effects were not life threatening. In individual cases it was necessary to modify the next dose of vaccine. The results confirm that specific immunotherapy is a safe method of treatment of correctly qualified patients suffering from atopic diseases. It is worth emphasizing that all patients in this study received allergen vaccines in professional medical settings and that they were under the close scrutiny of allergists during whole period of the treatment.

In this study we analyzed 224 patients. In total, 33 (13.7%) experienced local side effects, which occurred after 77 (1.6%) injections. The reactions like oedema (sized more than 5 cm) in the site of injection, pruritus or pain were qualified as late and safe symptoms, and resolved spontaneously or after applying antihistaminic drugs.

Other researchers have observed a higher incidence of LSEs of specific immunotherapy than in our study. Zenner et al. [7] analyzed safety of specific immunotherapy in patients suffering from IAR who were desensitized with allergoids of grass pollens. LSEs occurred after 9.7% of all injections. Frank et al. [8] reported early LSE after 0.6% of injections, and late LSE after 13% of injections in patients suffering from IAR. In another study [9] LSEs bigger than 5 cm occurred after 22.1% of injections in patients suffering from intermittent allergic rhinitis treated by depot vaccine containing allergoids of hazel, birch and alder pollens. LSEs were observed in the first year after 5.9% of injections, in the second year after 2.3% of injections and in the third year after 1% of injections, together after 189 (3%) among 6322 injections applied during three years.

Interestingly, Tamir et al. [10] indicated incidence of LSEs of 40% (average 3 LSEs per one patient) in patients suffering from inhalant allergy. Gawlik et al. [11] observed a significant decrease in number of local reactions like oedema bigger than 5 cm in successive years of immunotherapy of pollen allergy.

In the present study all systemic side-effects were late. These effects were noticed in 48 (21%) patients, after 1.29% of all injections. Administration of adrenalin was not necessary in any patient. Winter et al. noticed a similar frequency of SSEs (1.08% of injections) when usingAlutard in patients suffering from allergic rhinitis [12]. Rieckenberg et al. observed SSEs in 6.7% of 901 patients, and 1% of them needed administration of adrenalin. These reactions occurred in the maintenance phase and without any prodromal symptoms [13]. Zannino at el. evaluated risk of complications of injective IT in 1056 children aged 4-16 years. Mild systemic side-effects were observed in 3.7% of patients and one anaphylactic reaction [14]. Symptoms occurred more often in children treated with preparations containing house dust mite allergens than in those who received grass pollen extracts. Luigi et al. applied 300.086 injections of

allergens extracts to 6319 patients suffering from allergic rhinitis and/or asthma and observed only 0.061% SSEs (urticaria, mild aggravation of asthma and rhinitis) in 131 (2.1%) patients. Anaphylactic reactions did not occur [15]. It is hard to compare data concerning safety of IT because authors apply various vaccines and they use various methods of documentation. It is noteworthy that aqueous extracts of allergens used in the past were less effective and caused more side-effects as compared to extracts currently available [16-18].

Some authors draw attention to more frequent incidence of systemic side-effects in patients suffering from bronchial asthma and with high grade of sensitization. Stewart and Lockley [18] ascertained that the most dangerous were systemic side-effects occurring in less than 30 minutes after injection (so classified as early). These can precede anaphylactic reaction. The dose of vaccine should be increased carefully in patients who experienced dyspnoe after applying allergen vaccines. Kowalski et al. [19] recommended evaluation of peak expiratory flow value after applying each dose of vaccines in order to demonstrate bronchocontriction as early as possible

The same type of reactions in the same patients draws attention in the present study. It confirms the thesis that the incidence of side-effects during immunotherapy is individually variable. There was no relationship between SSEs and sex or age. In our study SSEs occurred in only 3 children under 10 years and in 3 adults above 35 years. Mean age of all patients experiencing SSEs was 25.1 years. Lin et al. [20] obtained similar results; they observed most SSEs in patients aged 16-39. Ragus et al. [21] also failed to show a relationship between systemic side-effects and sex or age, as well.

As in the present study more SSEs were observed after vaccines including grass pollens and in patients suffering from asthma, although 1/3 of all patients with SSEs suffered from IAR only. In Ragusa's study that lasted 10 years, systemic side-effects were less common during IT than whilst using pharmacotherapy. SSEs were noticed in 5.2% of patients after 0.06% of injections. This was in contrast to results by Buscino et al. [22] where SSEs occurred significantly more frequently in children desensitized with extracts of house dust mites than with extract of grass pollen. SSEs were observed in 3.7% of patients and after 0.09 % of injections. Karaayvaz et al. described 125 SSE in 109 patients among 1506 (8.3%) treated with aqueous extract of allergens for 12 years. Most of them (84.8 %) occurred early, in the maintenance phase (58.4%) and in the pollen season (60.8%). There was no correlation between SSEs and age, sex, incidence of asthma in desensitized patients [23].

In our study most complications occurred in the up-dosing phase, similar to the results obtained by others [13,23,24]. It is worth noting that 4 of our patients had side-effects after the very first dose of vaccine and 2 patients had SSE after 2 years of applying IT at monthly intervals. These practical valuable observations suggest that side-effects can occur whenever and irrespective of the concentration of allergens in preparation.

Concluding, analysis of our data confirmed that allergen immunotherapy conducted strictly according to the guidelines of EAACI is a safe method of treatment with only a few side-effects which are mostly mild in character.

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Quantitative analysis of nasal vascularization in allergic patients treated with mometasone furoate*

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SUMMARY

The purpose of this study was to compare vascularization of the nasal mucous membrane among non-allergic, non-treated allergic and allergic patients treated with mometasone furoate, by means of the stereology method in quantitative analysis. Three groups of patients (GP), each containing 10 patients were examined. The first group (GP 1) had a negative inhalatory allergen test while the second (GP 2) and third (GP 3) group both had positive results with the same test. GP 3 included allergic patients treated with mometasone furoate for 15 days before analysis, when a small piece of the nasal mucous membrane was taken from the frontal pole of the lower nasal shell. The specimens were examined immunohistochemically for expression of CD31 and VEGF-C. Vascular phase was determined by using length density (L_v). Differences in CD31 and VEGF-C expression were compared using one-way ANOVA and Tukey HSD post-hoc tests. CD31 expression in GP 1 had significantly lower values than in the GP 2 and GP 3 groups ($p < 0.001$). VEGF-C expression in GP 1 was significantly lower than in GP 2 ($p = 0.007$), but not in GP 3 ($p = 0.292$). We have shown that 15-day treatment with mometasone furoate results in a significant reduction of the density of vascular elements in allergic patients.

Key words: vascularization, nasal mucous membrane, allergy, mometasone furoate, stereology

INTRODUCTION

Angiogenesis plays an important role in diverse pathological mechanisms. It has been suggested that vascularization of the nasal mucous membrane in patients with allergies differs from that found in patients with healthy mucosa and that changes in the vascular network contribute to the pathogenesis of allergic rhinitis [(1)]. An increased number of blood vessels could be one of the factors influencing the overall increase in the surface of the blood vessel walls followed by increased permeability as well as transition of a greater number of eosinophiles and other inflammatory cells into the surrounding tissues. The greater the number of vessels and subsequently their wall permeability, the likelihood of more intensive inflammatory reaction increases. Thus, the question is whether local medicament therapy (mometasone furoate) of allergic patients could lead to a substantial change in nasal mucous membrane vascularization.

MATERIALS AND METHODS

Patients

For the purpose of this study, three groups of patients (GP), each consisting of 10 patients were tested. The patients were grouped according to the results of an allergy test to inhalatory allergens, including: grass, weed, tree pollen, feathers, herbal fibbers, fungi, fabric, animal hairs, domestic dust, dermatophagoides pteronyssimus and bacteria. The first group (GP 1) (patients without allergies; with a negative allergic reaction to all of the allergens) included four female and six male patients, age range from 23 to 47 years (average age 31.5 years). The second group (GP 2) (allergic patients; with a positive allergic reaction to one or more allergens) included three female and seven male patients, age range from 18 to 37 years (average age 27.5 years). The third group (GP 3) (allergic patients treated with mometasone furoate) consisted of five female and five male patients, age from 18 to 46 years (average age 31.9 years). Signed informed consent was obtained from all patients before the procedure. This study has been approved by the Ethics Committee of the School of Medicine, University of Rijeka.

Immunohistochemistry and vascular density analysis

A small piece of the nasal mucous membrane, size 5x5 mm, was excised from the lower nasal shell and used for the analy-

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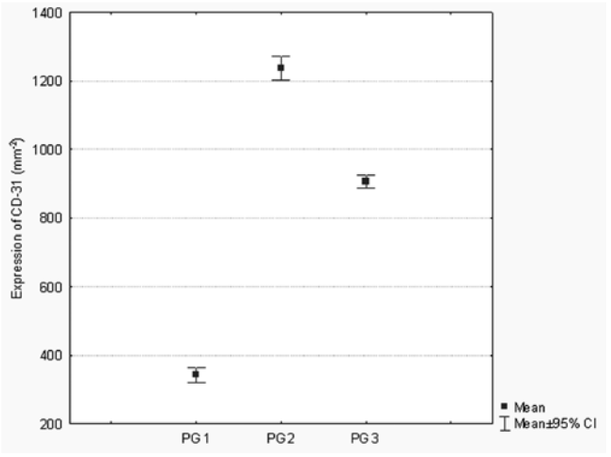

Figure 1. Expression of CD31 in GP 1 (patients without allergies), GP 2 (patients with allergies), and GP 3 (patients with allergies treated with mometasone furoate), ($p<0.001$*).
* Significant difference between all three groups

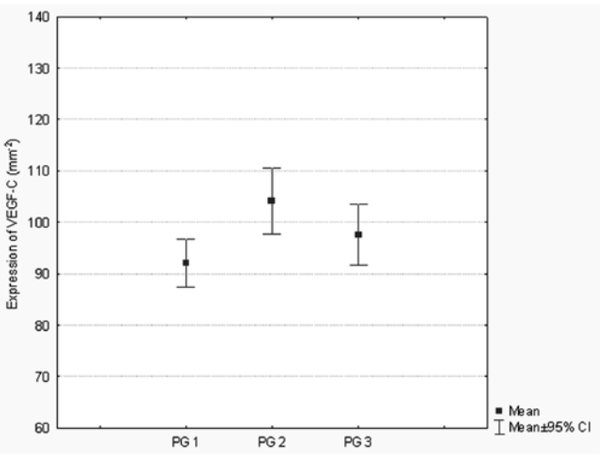

Figure 3. Expression of VEGF-C in GP 1 (patients without allergies), GP 2 (patients with allergies), and GP 3 (patients with allergies treated with mometasone furoate); ($p=0.010$*).
* Significant difference GP 1 vs. GP 2

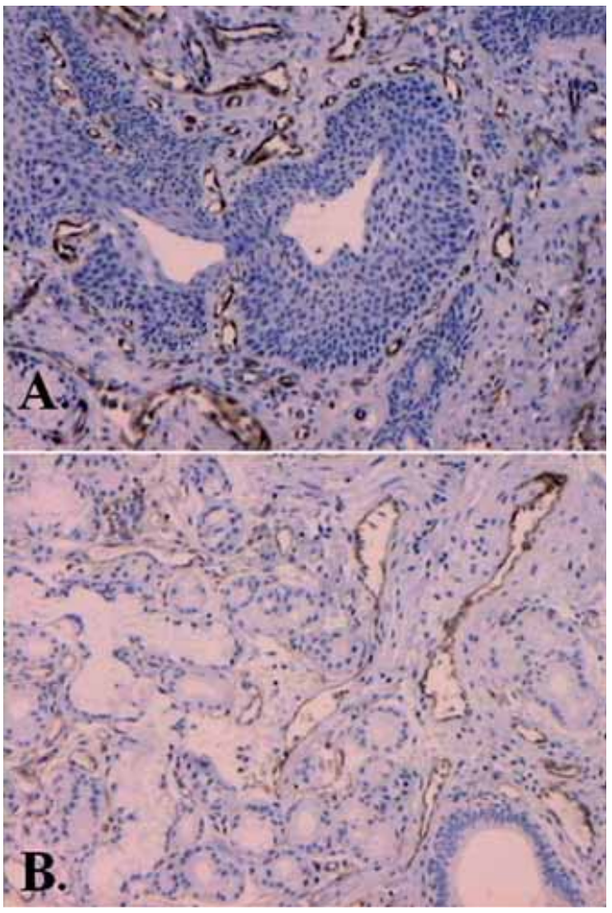

Figure 2. Immunohistochemical staining for CD31. A) In patients with allergy there was a mucous oedema and high number of CD31 positive vascular spaces. B) In patients treated with mometasone furoate there was a significant reduction of epithelial oedema and CD31 positivity. Magnification x200.

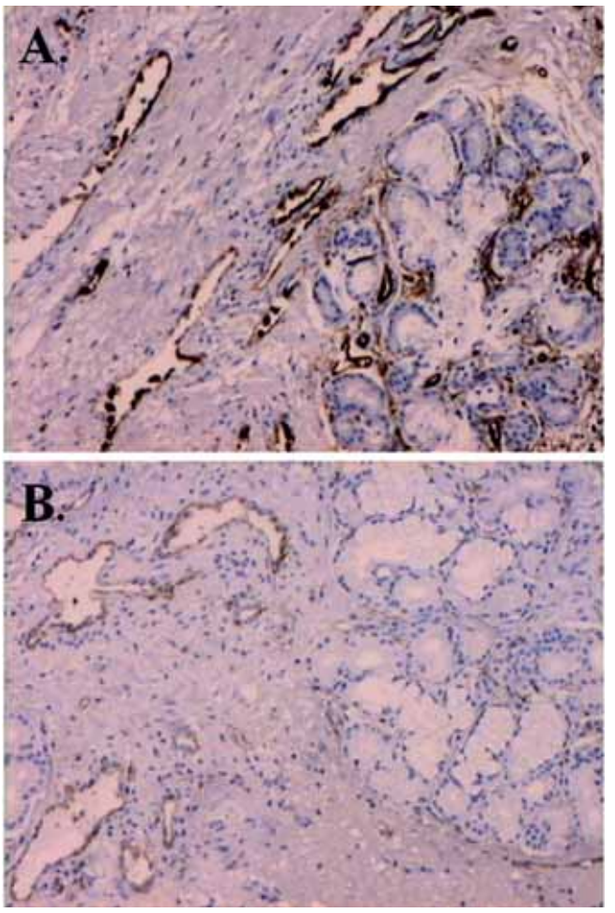

Figure 4. Immunohistochemical staining for VEGF-C. A) In patients with allergy there was a mucous oedema and high number of VEGF-C positive lymph capillaries. B) In patients treated with mometasone furoate there was a significant reduction of epithelial oedema and VEGF-C positivity. Magnification x200.

sis. After excision the mucous membrane was formalin fixed, paraffin embedded and 4 μm thick sections were cut. All sections were examined immunohistochemically for the expression of CD31 (1:50, clone JC 70A, DAKO A/S, Glostrup, DK) and VEGF-C (1:100, clone F 8/86, DAKO) [2-5].

The vascular phase of the nasal mucous membrane was determined by means of the stereological method using the length density variable. Length density is a relative stereological variable that gives us the length of a certain curve in a unit of volume. The property of length density has an exponent of -2 ($cm^1/cm^3 = cm^{-2}$ - our research made use of mm^{-2}). The trace of a one-dimensional curve in a plane is a dimensionless value called a transection point, marked Q. Its density in the test plane is referred to as density transection (Q_A). The formula employed to calculate the length density was $L_{vf} = 2 \times Q_f / A_t$); where Q_f in the formula stands for the number of transections, and At for the test plane surface, which in our case equals 0.013 mm^2. During the study approximately 100 measurements per specimen were taken and mean values were calculated for each sample.

Statistical analysis

Results are presented as the mean ± standard deviation. We used one-way ANOVA and Tukey HSD as a post-hoc test to compare differences in CD31 and VEGF-C expression between all three groups. All statistical values were considered significant at the p level of 0.05. Statistical analyses of data were performed by Statistica for Windows, release 6.1 (Stasoft, INC., Tulsa, OK, USA).

RESULTS

Values of CD31 expression in all GPs are shown in Figure 1. Histologically there was a significant decrease of microvasculature (blood capillaries) in allergic patients following mometasone therapy (Figure 2A and B). Statistical analysis of data showed that differences in CD31 values were significant at the level $p < 0.001$ in all comparisons. We detected a significant increase in CD31 expression in the GP 2 group (allergic non-treated patients) when compared to the other two GP's. Also, there was a significantly higher CD31 expression in GP 3 (treated allergic patients) compared to the non-allergic patients (GP 1).

Values of VEGF-C expression in all GPs are shown in Figure 3. Histologically there was a significant decrease in the number of lymph capillaries in allergic patients following mometasone therapy (Figure 4A and B). There was a significant increase in VEGF-C expression between GP 1 and GP 2 ($p = 0.010$). VEGF-C expression was increased in GP 3 compared to GP 1 although the difference did not reach statistical significance ($p = 0.292$).

DISCUSSION

Treatment of the group of allergic patients with mometasone furoate [6], which has a corticosteroid effect, 15 days before the surgical procedure, confirmed our assumption that the drug, besides its major effect in the late phase of inflammation (inhibition of interleukin release and other cytokines together with inhibition of the synthesis of leukotriens, which reduce vascular permeability) also reduces the number of blood vessels in the nasal mucous membrane. Mometasone furoate is powerful synthetic corticosteroid with a wide range of use as an anti-inflammatory agent for reducing seasonal and permanent allergic rhinitis [7-9]. Its glucocorticoid anti-inflammatory effect has minimal system activity and *in vitro* experiments have shown inhibition of pro-inflammatory Th2 cytokines [6]. It is known that mometasone furoate has an effect on nasal glucocorticoide receptors and inhibits leukotrien synthesis. Inhibition of leukotriens synthesis causes reduced permeability of the blood vessels [10, 11]. The intensity of the allergic reaction is, therefore, proportional to the permeability of the blood vessels. Thus, it is logical to assume that along with the permeability of the blood vessels for the intensity of the allergic reaction, the density of the blood vessels is of equal importance. Obtained results indicate that after a 15-day treatment with mometasone furoate the nasal mucous membrane of allergic patients showed significant reduction of the density of vascular elements. These effects are considered to be additional effects of the drug in the treatment of allergic diseases involving the upper respiratory system mucous membrane.

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The relationship between subjective assessment instruments in chronic rhinosinusitis*

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SUMMARY

Problem: *To provide an evidence-based definition for the relationship between three subjective instruments for assessing severity of chronic rhinosinusitis- visual analogue scale, 'mild', 'moderate' and 'severe' (MMS) classification and perception of whether quality of life (QoL) is affected.*

Method of study: *One hundred sixteen subjects with chronic rhinosinusitis filled in a questionnaire rating simultaneously their perception of disease severity based (1) upon visual analogue scale, (2) MMS classification and (3) stating whether they felt their QoL was affected.*

Main results: *The mean age of subjects was 50. The inter-quartile range of VAS scores for the respective MMS groups were: Mild 0.80-3.50, Moderate 4.40-6.33, Severe 7.70-9.50. In the group who perceived effects on QoL, the inter-quartile range for VAS scores was 5.10-8.68. This range was 1.53-4.57 in the other group with no effects on QoL. 30.3 % of patients in the 'mild' category, 79.6% in the 'moderate' category and 97.4% in the 'severe' category felt that their QoL was affected.*

Principle conclusions: *We propose a statistically validated definition of the relationship between MMS classification and VAS scoring. Based on our study we would define 'mild' as being 0- 3 inclusive, 'moderate', as >3- 7 inclusive and 'severe' as >7- 10 inclusive on the VAS scoring system. We further propose that in general QoL is more likely to be affected with VAS scores of 5 or more.*

Key words: Visual Analogue Scale, 'mild', 'moderate', 'severe', quality of life, chronic rhinosinusitis

INTRODUCTION

Chronic rhinosinusitis is a common condition, affecting about approximately 14% of the population [1]. It significantly affects health [2] and has a considerable economic burden upon society [3].

The diagnosis of chronic rhinosinusitis involves fulfilling historical and/or endoscopic and radiological criteria [4]. Classification of severity of disease, as with most other diseases is less standardised and a variety of methods exist. Both objective and subjective methodology may be used. Subjective methods include the tri-categorical classification of 'mild', 'moderate' and 'severe' (MMS), Visual Analogue Scales (VAS) and Quality of Life evaluation (QoL). Employing more than one method may improve accuracy in determining disease severity.

The EPOS document [4] has arbitrarily classified VAS 0-4 mild and 5-10 moderate/ severe. We sought to statistically validate this classification and also to examine the relationship between the (a) VAS score and QoL (b) mild/moderate/severe (MMS) classification and QoL.

MATERIALS AND METHODS

In total, 118 consecutive patients attending clinic for treatment of chronic rhinosinusits participated. Subjects were asked to fill in a questionnaire in which they (a) rated their overall symptoms of chronic rhinosinusitis on a VAS scale, (b) categorised their overall symptoms as 'mild', 'moderate' or 'severe' (c) indicated whether their symptoms affected the quality of their life. Only 2 patients rated their overall symptoms on the VAS scale as 0 and were excluded from the study.

Statistical analysis

Statistical analyses were performed with Statistical Package for the Social Sciences (SPSS, version 14.0 for windows, SPSS Inc., Chicago, IL, USA). The relationship between (a) VAS and MMS classification and (b) VAS and QoL were examined using box plots showing the median values and the upper and lower quartiles. Receiver Operating Characteristic (ROC) curve

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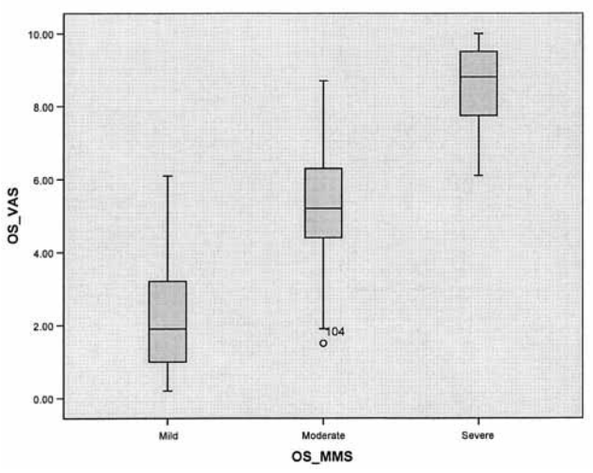

Figure 1. Box and whisker plot for MMS and VAS scores.

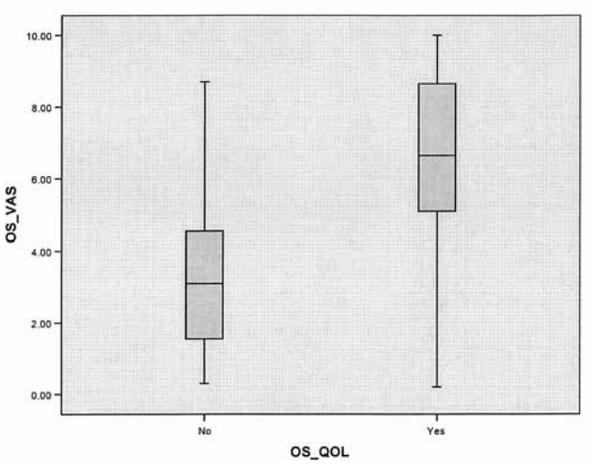

Figure 2. Box and whisker plot for QoL and VAS scores.

Table 1. MMS and VAS scores.

VAS scores	Mild	Moderate	Severe
Lower extreme	0.2	1.9*	6.1
25th percentile	0.8	4.4	7.7
Median	1.9	5.2	8.8
75th percentile	3.5	6.3	9.5
Upper extreme	6.1	8.7	10

*with a single off scale value of 1.5

was employed in further analysing the relationship between VAS and QoL.

RESULTS

Demographics and overall results

Of the 116 patients analysed, 58 were male and 58 were female. The age ranged from 10 to 81 and the mean age was 50. Twenty-three patients rated their symptoms as mild, 54 as moderate and 39 as severe. The VAS scores ranged from 0.2 to 10, with a mean VAS score of 5.8. A total of 88 subjects described their symptoms as affecting their quality of life.

VAS scores and MMS

As expected, the median VAS values varied between the 3 MMS categories. Table 1 and Figure 1 illustrate the median values, upper/ lower quartiles and extreme values of the VAS scores for the 3 categories of MMS.

The inter-quartile ranges for the respective MMS groups were: Mild 0.80-3.50, Moderate 4.40-6.33, Severe 7.70-9.50. Median values for the respective MMS groups were: Mild 1.9, Moderate 5.2, Severe 8.8.

VAS scores and QoL

For the group in which QoL was affected the reported VAS scores had a median of 6.65 and an inter-quartile range of 5.10-8.68. The group of subjects in which QoL was not affected had VAS scores with an inter-quartile range of 1.53-4.57 and a median value of 3.1 (Figure 2).

MMS and QoL

Seven out of 23 subjects in the 'mild' category felt that their quality of life was also affected. Quality of life was affected in 43 out of 54 patients in the 'moderate' group and 38 out of 39 patients in the 'severe' group

ANALYSIS

VAS scores and MMS

Based on the inter-quartile results of our study and employing whole numbers only for simplicity two alternative classifications for definition may now be proposed:

(1) 'Mild' being defined on the VAS as 0- 4 inclusive, 'moderate' as > 4- 7 inclusive and 'severe' as > 7- 10 inclusive. Using this definition means that 20/29 = 69% patients in our study with a score of 0-4 also classified their symptoms as 'mild', 40/46 = 87% of patients with a score of > 4-7 classified their score as 'moderate' and 35/40 = 87.5% of subjects with a score of > 7-10 classified their score as 'severe'.

(2) 'Mild' being defined on the VAS as 0- 3 inclusive, 'moderate' as > 3- 7 inclusive and 'severe' as > 7-10 inclusive. Using this definition 17/20 = 85% patients in our study with a score of 0-3 classified their symptoms as 'mild', 46/55 = 83% of patients with a score of > 4-7 classified their symptoms as 'moderate' and 35/40 = 87.5% of subjects with a score of > 7-10 classified their symptoms as 'severe'.

It is clear that the second definition significantly increases the correlation between VAS scoring and the MMS tri-categorical classification in our study. The increase in correlation is most marked for the 'mild' category.

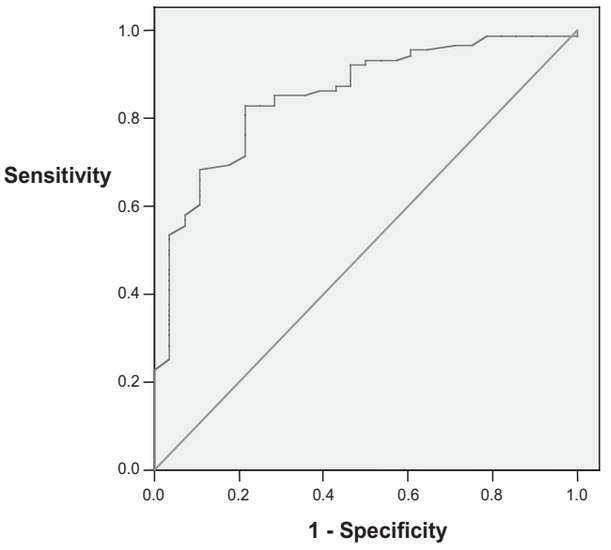

Figure 3. ROC plots for QoL and VAS scores.

VAS scores and QoL

In examining the relationship between QoL and VAS scores, ROC analysis shows that VAS scoring is a good discriminator of whether QoL is affected (area under curve 0.853, 95% CI 0.776-0.930, Figure 3) The definition with the best performance was when VAS was dichotomised at 4.75 generating a sensitivity of 83% and specificity of 78.6%.

MMS and QoL

Examining the relationship between MMS categorisation and QoL reveals that 30.3 % of patients in the 'mild' category, 79.6% in the 'moderate' category and 97.4% in the 'severe' category feel their QoL is affected.

DISCUSSION

Defining symptom severity is important for several reasons. It aids clinicians in the decision making process of choosing appropriate pharmacotherapy. It also allows evaluation of success of therapy and aids the advancement of research.

A variety of subjective and objective methods for classifying symptom severity in chronic rhinosinusitis exist. For subjective classification methods the Sinonasal Outcome Test-22 (SNOT-22) has been shown to be the best available methodology based on its reliability, validity and responsivness [5]. However, use of this instrument may be time-consuming and hence may not be practically feasible in routine clinics. Instead, employing simple subjective instruments such as VAS scoring, MMS and QoL may be more feasible in obtaining a quick and simple understanding of severity of disease and response to medication.

Because these three simple instruments may not always be used concomitantly, it is useful to obtain some idea of the relationship between them. The EPOS document [4] has defined symptom scores of 0-4 on the VAS as mild and 5-10 as moderate/ severe.

There are 3 potential problems with this definition. Firstly the definition is arbitrary rather than based on validated epidemiological studies. Secondly, no differentiation is made between the 'moderate' and 'severe' categories. Finally, the definition does not allow for a continuous range of VAS scores with scores between 4-5 unsatisfactorily left without classification. The problem arises as the VAS scale normally has no demarcations along it [6], inevitably generating non whole numbers when employed.

Our analysis proposes the evidence-based classification of 'mild' being defined on the VAS as 0-3 inclusive, 'moderate' as > 3-7 inclusive and 'severe' as > 7-10 inclusive. There are 3 advantages of this definition: (a) It is a statistical construct based upon an epidemiological study, (b) it is a definition that allows differentiation between 'moderate' and 'severe' categories and (c) it provides an appropriate classification for the continuous range of scores the VAS instrument generates. We would propose this as a more suitable definition than what has been arbitrarily defined in EPOS [4].

Nonetheless it is important to be aware that there will always be a small percentage of patients who do not obey the definition proposed. Hence it may be prudent to obtain for each patient both VAS and MMS scores to improve accuracy of overall global subjective assessment. In addition it is realised that the proposed definition is based upon a small dataset, and that further larger studies might be appropriate.

In analysing the relationship between VAS scores and QoL we find good corroboration between ROC analysis and inter-quartile range analysis. Based on both these analyses we propose that in general QoL is more likely to be affected with VAS scores of 5 or more. It will be noted however that 17 out of the 88 patients (19%) who felt their quality of life to be affected gave a VAS score less than 5, so again there is a proportion of patients who will not fulfil this definition.

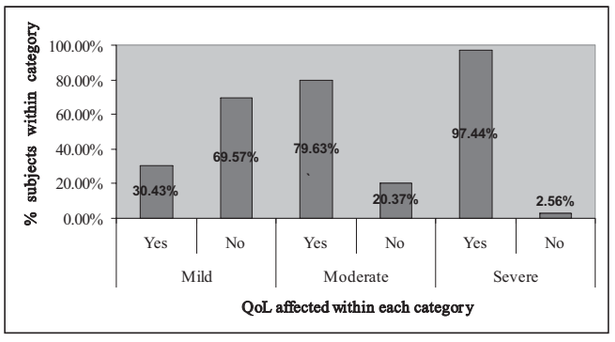

Figure 4. Percentage of subjects QoL is affected in each MMS category.

Exploring the relationship between MMS categorisation and QoL reveals that there is an expected increase in percentage of subjects in whom QoL is affected in moving from the mild to severe categories. However it is clear from our analysis that individual perception of effects on QoL is not always proportional to perceived severity of symptoms. Nearly one-third of all patients who described their symptoms as 'mild' also perceived an effect on their quality of life. This highlights the importance of eliciting effects on quality of life as well and not severity of illness only during consultation to gain a balanced understanding of disease impact.

CONCLUSION

Our study provides a statistically based definition of the relationship between VAS scoring, MMS classification and QoL perception. Based on our study we would define 'mild' as being 0-3 inclusive, 'moderate', as > 3-7 inclusive and 'severe' as > 7-10 inclusive on the VAS scoring system. This is in contrast to the current definition arbitrarily proposed in the EPOS document [4]. We further propose that in general QoL is more likely to be affected with VAS scores of 5 or more. Further larger studies might be useful to validate our proposed definitions.

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Rhinology, 45, 148-152, 2007

Histological structure of the nasal cartilages and their perichondrial envelope
I. The septal and lobular cartilage*

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SUMMARY

The cellular elements and extracellular matrix of the nasal septal cartilage and the lateral crus of the lobular cartilage were studied in serial coronal sections of five human cadaver noses. To discern the various tissue components, the sections were stained according to the methods of Mallory-Cason, Azan, Herovici, Verhoeff-van Gieson, and Lawson as well as by immunohistochemistry to demonstrate the presence of collagen type I and II.

A characteristic gradual transition of the chondrocytes was observed in both septal and lobular cartilage: from numerous small flat cells oriented parallel to the surface of the cartilage to less numerous larger ovaloid cells oriented perpendicular to the surface. This difference between the peripheral and central zones of the cartilage was particularly marked in lobular cartilage.

Both septal and lobular cartilage have a high density of type II collagen but almost none of type I. The peripheral zones of the matrix showed a higher density of collagen than the central zone. This difference was more pronounced in septal than lobular cartilage. The high density of type II collagen in septal cartilage, particularly in the peripheral zones, suggests that one of the primary tasks of the septum is providing stiffness to the external nose. That idea is consistent with findings from our study of the perichondrial envelope.

INTRODUCTION

The framework of the human nose is comprised of five main cartilaginous elements: the septal cartilage; two triangular cartilages (together constituting the septolateral cartilage); and two lobular cartilages. In addition, there are various small sesamoid cartilages in the intercartilaginous joint areas and two or three accessory cartilages in the lateral soft-tissue areas [1,2].

These cartilaginous structures are made up of hyaline cartilage, consisting of chondrocytes and an extracellular matrix (ECM). The chondrocytes have the capacity to synthesize the cartilage matrix. Some have short cilia extending into the matrix. There are no contacts between the individual cells. The matrix consists of water (80%) and a macromolecular framework of collagens, proteoglycans, and non-collagenous proteins, respectively amounting to 60, 25-35, and 15-20% of its dry weight. Multiple types of collagens are present, in particular types II (90-95%), IX, and XI, which form the cross-banded fibrils that provide stiffness [3,4]. In studies of articular cartilage, Buckwalter and Mankin distinguished four layers or zones with different morphological and functional features [3].

Cartilage has a low-level metabolism. Besides lacking vascular and nerve supply, it has no healing capacity, which has major implications for nasal pathology and surgery [5,6].

The nasal cartilaginous framework provides the external nasal pyramid with a degree of stability and mobility. The present study examines the morphology and arrangement of cells and the composition of the matrix in these cartilages.

MATERIAL AND METHODS

Staining of specimens

We studied five human noses from cadavers of Caucasian origin aged 68, 75, 75, 80, and 87 years.

The specimens were preserved and fixed in 4% buffered formaldehyde, decalcified with sodium formiat solution, and then dehydrated in increasing concentrations of alcohol and embedded in paraffin. Serial sections of 10, 20, and 25μm thickness were cut in the coronal plane at intervals of 200 and 400μm. They were mounted on glass slides and stained according to the following methods. A modified Mallory-Cason trichrome stain was used to discriminate between bone, cartilage, and connective tissue [7]. Azan stain was applied to visualize collagen fibers and Herovici stain to discriminate between young and mature collagen. Verhoeff-van Gieson stain was used to demonstrate elastic and collagen fibers, whereas Lawson stain was used to demonstrate the presence of elastic fibers.

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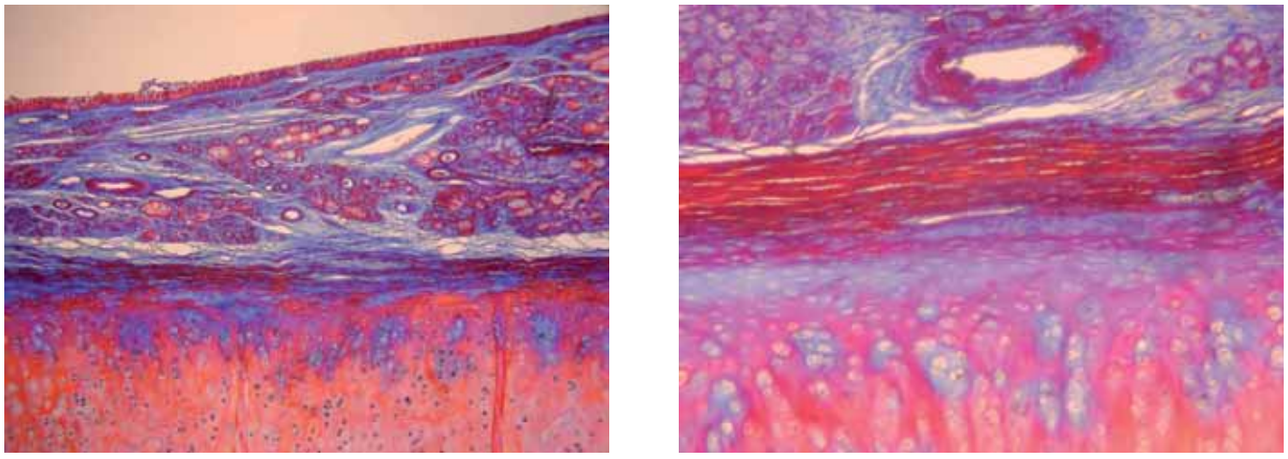

Figure 1. Septal cartilage with perichondrium and mucosa (Mallory-Cason staining). Left: overview, right: detail. In the peripheral zones of the cartilage the chondrocytes are numerous, small, flat, and oriented parallel to the surface of the cartilage. In the more central zones they are larger, more round, clustered, and oriented perpendicular to the surface. The bluish staining of the matrix in certain areas of the peripheral zones and around some of the chondrocytes indicates a rich collagen content.

Immunohistochemistry for types I and II collagen was performed by the ABC method at room temperature. The sections were washed in PBS and pretreated with 1% H_2O_2. They were washed again in PBS, followed by pre-incubation in 5% goat serum (type I) or 5% rabbit serum (type II) in PBS for 60 minutes. Subsequently, the sections were incubated overnight in anti-collagen I antibodies (Abcam plc., Cambridge, U.K.) or anti-collagen II antibodies (Chemicon International Inc., Temecula, CA, U.S.A.) diluted 1:400 or 1:100 respectively in PBS containing 2% bovine serum albumin (BSA). After washing in PBS, the specimens were incubated for 60 minutes in goat-anti-rabbit IgG biotin (type I) or rabbit-anti-mouse IgG biotin (type II) (Dako, Glostrup, Denmark) diluted 1:600 in PBS containing 2% BSA. The sections were washed in PBS and subsequently incubated for 30 minutes in ABC complex (Dako, Glostrup, Denmark) diluted 1:800 in PBS. The sections were washed in PBS and subsequently in 0.01 M sodium acetate. After incubation for 5 minutes in 0.04 % diaminobenzidine and 0.01% H_2O_2 in 0.01 M sodium acetate, the sections were washed in PBS and mounted in Entellan (Merck, Darmstadt, Germany).

RESULTS

Septal cartilage

Cellular elements: Chondrocytes, which are the only cellular elements in septal cartilage, are clearly demonstrated by the Mallory-Cason staining (Figure 1). They differ in size and shape but also in metabolic activity. Based on these differences, we can distinguish three zones and a gradual transition between them. In the peripheral zones, we find the young cells. They are numerous, small, flat, and oriented parallel to the surface of the cartilage.

Cells in the intermediate zones are less numerous and more ovaloid. Their axis runs more perpendicular to the cartilaginous surface.

The lowest density of chondrocytes is found in the central zone. Here, the cells are spheroidal and more or less aligned in columns perpendicular to the cartilaginous surface.

Extracellular matrix: The extracellular matrix of septal cartilage shows distinct differences in composition between the peripheral and central zones. The small and flat young cells in the peripheral zones are surrounded by a homogeneous light-blue staining material, suggesting a rich collagen content (Figure 1). In the intermediate zones, the matrix stains more pinkish. In the central zone, the chondrocytes are surrounded by more or less blue-staining material, whereas the remaining matrix stains pink to red.

With the Azan staining, the peripheral zones appear bluish, in contrast to the more central zones that stain more reddish (Figure 2). This confirms the presence of a higher density of collagen fibers in the outer areas.

Using Herovici staining, the matrix of the peripheral zones stains light blue, characteristic of young collagen, whereas the matrix of the central zones has a more reddish color, specific

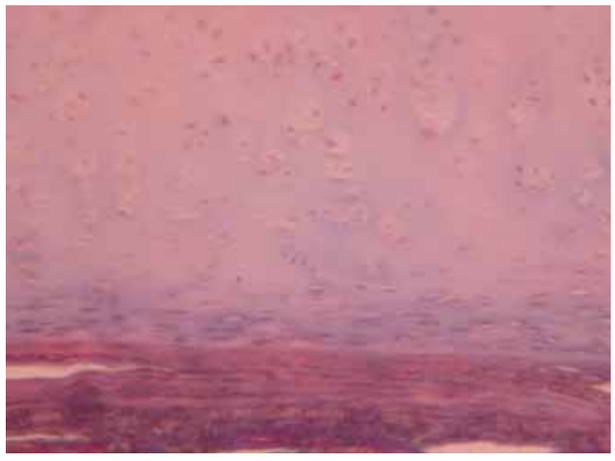

Figure 2. Septal cartilage with perichondrium (Azan staining). Bluish color of peripheral matrix indicates a high density of collagen fibers.

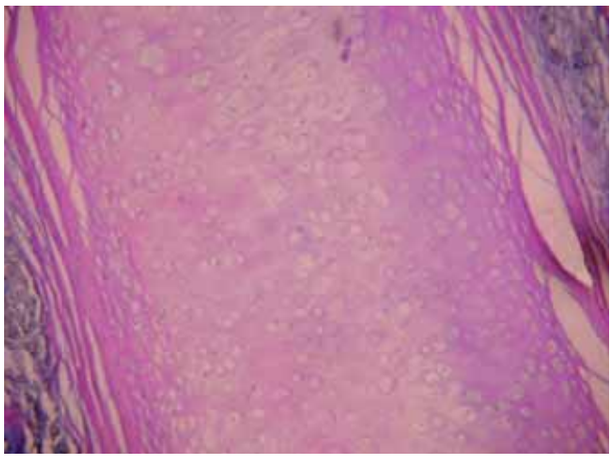

Figure 3. Septal cartilage with perichondrium (Herovici staining). Light-blue coloring of the matrix in the peripheral zones demonstrates the presence of young collagen.

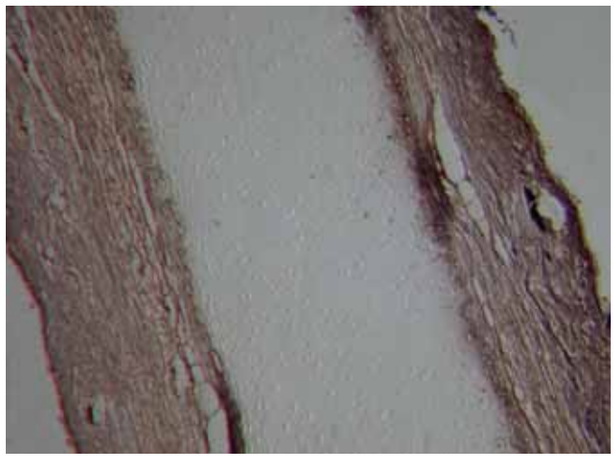

Figure 4. Septal cartilage with perichondrium and mucosa (type I collagen staining). Abundance of dark-brown staining type I collagen fibers can be seen in the perichondrium. No type I collagen present in the cartilage.

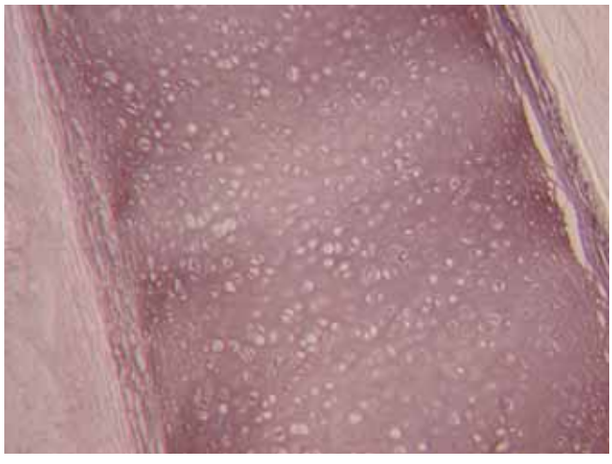

Figure 5. Septal cartilage with perichondrium (type II collagen staining). Collagen type II is observed in all zones of the cartilaginous matrix. Several somewhat darker staining areas reflecting a somewhat higher collagen II concentration are seen in the peripheral zones.

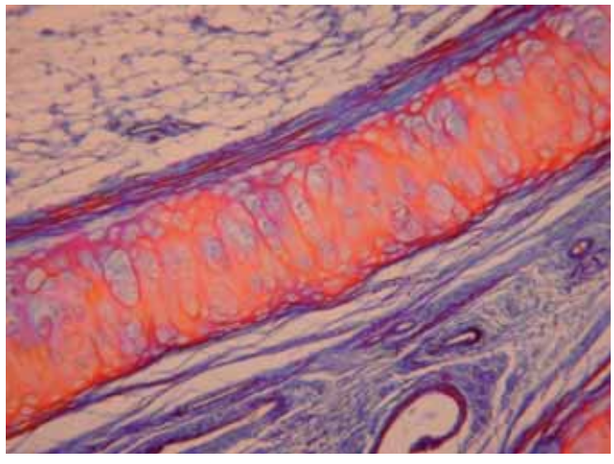

Figure 6. Lateral crus of lobular cartilage with perichondrium and adjacent connective tissue (Mallory-Cason staining). The morphology and orientation of the chondrocytes in the peripheral and central zones of the cartilage are similar to those in the septal cartilage. There is a distinct difference in the staining between the matrix of the septal cartilage and that of the lobular cartilage.

to mature collagen (Figure 3).
Immunohistochemistry staining for type I collagen reveals its complete absence in the cartilage but its high content in the perichondrium (Figure 4).
Type II is observed in all zones of the septal cartilage, with a somewhat higher concentration in certain peripheral areas (Figure 5).
Staining according to the methods of Verhoeff-van Gieson and Lawson does not reveal any elastic fibers in the septal cartilage but does demonstrate their presence in the septal perichondrium [8].

Lobular cartilage

Findings in the lateral crus of the lobular cartilage resemble those in septal cartilage in many respects. However, in the lobular cartilage the difference between the small flat chondrocytes lying parallel to the surface in the peripheral zones and the larger cells arranged palisade-like perpendicular to the cartilage surface in the central zones is more distinct (Figure 6).
Herovici staining demonstrates the presence of young ECM in the peripheral zones (staining bluish) (Figure 7).
Histochemistry on both type I and type II shows an absence of collagen I and a high content of collagen II in the cartilaginous matrix. These results are similar to the findings in septal cartilage is more distinct (Figures 8 and 9).

DISCUSSION

Most of the research on cartilage has been performed on articular cartilage. An excellent overview of the results was published by Buckwalter and Mankin [3]. They distinguished four different zones in articular cartilage. In the present study of the nasal cartilages, looking at cell morphology and arrangement

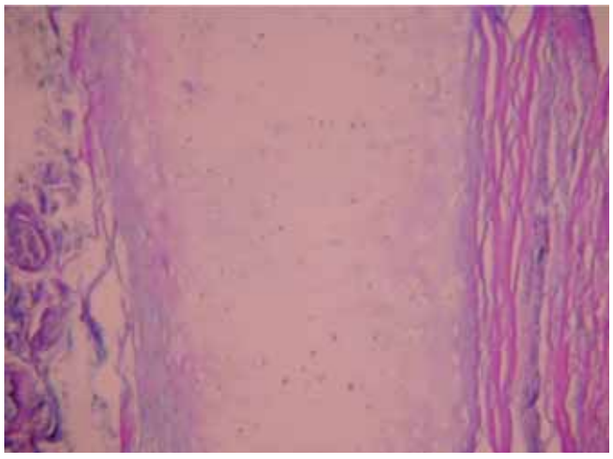

Figure 7. Lateral crus of the lobular cartilage with perichondrium (Herovici staining). The matrix of the peripheral zones stains bluish, indicating the presence of young collagen.

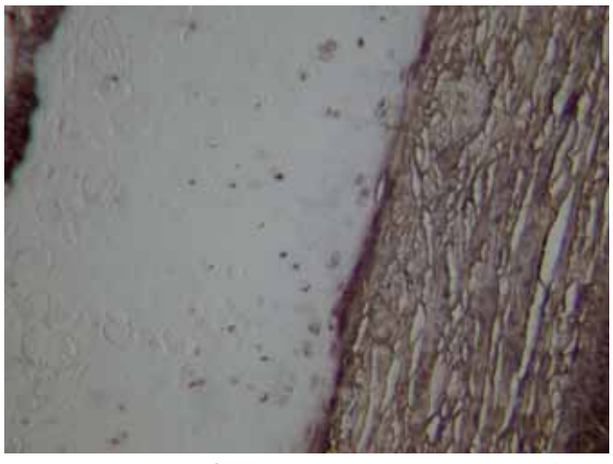

Figure 8. Lateral crus of lobular cartilage with perichondrium (type I collagen staining). High density of collagen I in the perichondrial fibers, no collagen type I in the matrix.

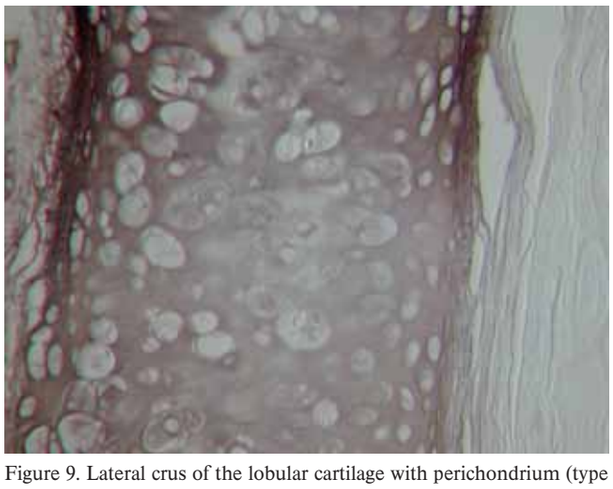

Figure 9. Lateral crus of the lobular cartilage with perichondrium (type II collagen staining). Collagen type II is observed in all zones of the cartilaginous matrix, particularly in the outermost zones.

as well as matrix composition, we found three zones that are consistent with the outer zones in articular cartilage. The fourth zone found in articular cartilage is related to the transition between cartilage and bone and is thus missing in nasal cartilage.

In both septal and lobular cartilage, we found a distinct transition from numerous small flat chondrocytes lying parallel to the cartilaginous surface to less numerous and larger ovaloid cells lying perpendicular to the surface. These differences represent the change in form and position between younger and mature cells. We cannot explain why the transition from small flat cells to larger ovaloid ones is accompanied by a 90-degree shift in cell orientation. Nor do we know why this association is more pronounced in the lateral crus of the lobular cartilage than in the septum. One could speculate that this shift in orientation is related to a special function of nasal cartilages, namely to provide a resistant yet pliable framework. Clearly, support is the primary function of septal cartilage. Lobular cartilage should have enough rigidity to keep the vestibule and the nasal valve area open, but at the same time it should allow mobility of the lateral nasal wall during respiration.

The extracellular matrix of both the septal and lobular cartilage was found to have a high density of type II collagen but no type I, as shown by our histochemical methods. This outcome is in agreement with observations made by others (3,4).

Like Üstünel et al.,(4) we found distinct differences between the peripheral and central zones, particularly in septal cartilage. Azan and Verhoeff-van Gieson staining demonstrated that the highest density of collagen is present in the peripheral zones. When applying Herovici and Lawson staining, the collagen in these outer areas appeared to contain more young collagen than the central zones, as was to be expected. Verhoeff-van Gieson and Lawson staining did not show elastic fibers in the cartilage.

In the matrix of the lobular cartilage, similar differences were found, although to a lesser degree. The high density of type II collagen in the septal cartilage, especially in the peripheral zones, supports the idea that one of the primary tasks of the septum is to provide stiffness to the external nose.

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Histological structure of the nasal cartilages and their perichondrial envelope
II. The perichondrial envelope of the septal and lobular cartilage*

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SUMMARY

The perichondrial envelopes of the septal cartilage and the lateral crus of the lobular cartilage were studied in serial coronal sections of five human noses. To differentiate between the various tissue components, the sections were stained according to Mallory-Cason, Azan, Herovici, Verhoeff-van Gieson, and Lawson. Collagen types I and II were immunohistochemically stained. The results demonstrated that the perichondrium of the septal cartilage and the lateral crus of the lobular cartilage consists of a homogeneous layer of type I collagen fibers and elastic fibers. The elastic fibers have a network-like arrangement and are most numerous in the perichondrium of the lateral crus of the lobular cartilage. Clearly distinguishable zones in the perichondrial envelopes could not be observed. The perichondrium on the outside of the lateral crus of the lobular cartilage and the triangular cartilage is significantly thicker than the inner perichondrium. It is speculated that these morphological characteristics of the perichondrial envelopes are related to functional differences between the cartilages. The mobility of the lateral crus of the lobular cartilage requires a higher content of elastic fibers in its perichondrium than the more rigid septal cartilage. A thicker outer perichondrium of the lateral crus of the lobular cartilage and the triangular cartilage may be related to muscular forces that are exerted on the outer side of the cartilages only.

INTRODUCTION

The perichondrium is a dense connective tissue layer that covers mammalian cartilage except at articular surfaces, where cartilage is exposed to synovial fluid. Recognizing that the perichondrial structure varies with cartilage location, it is suggested that the variation is related to differences in functional requirements. Nevertheless, a general structural plan in which three zones may be distinguished has been proposed: 1. a loose outer layer containing blood vessels and nerve fibers; 2. a main layer of dense connective tissue; and 3. an internal zone of fusion with the extracellular matrix (ECM) of the underlying cartilage [(1)]. The internal zone is not recognized as a separate layer by all authors [(2)].

The perichondrium consists of cellular elements – the perichondrocytes, which are fibroblasts – and the ECM. Collagen and elastic fibers are found in the ECM, which is synthesized by perichondrocytes. The quantity and orientation of the fibrous elements varies according to the cartilage type and site [(1)]. Detailed descriptions of the perichondrium of particular nasal cartilages are still lacking, however. Considering the functional differences between septal and lobular cartilages, as discussed in the accompanying article by Popko et al., [(3)] the corresponding perichondria may demonstrate variations in morphology. The present study examines the morphological details of the perichondrium of human septolateral and lobular cartilages.

MATERIAL AND METHODS

Staining of specimens

Five human noses from cadavers of Caucasian origin aged 68, 75, 75, 80, and 87 were studied.

The specimens were preserved, fixed, and sectioned as described in the accompanying article [(3)]. Several staining methods were applied. A modified Mallory-Cason trichrome stain [(4)] was used to discriminate between bone, cartilage, and connective tissue. Azan stain was applied to visualize collagen fibers, Herovici stain to discriminate between young and mature collagen. Verhoeff-van Gieson stain was used to demonstrate elastic and collagen fibers, Lawson stain to demonstrate elastic fibers. Type I and type II collagens were immunohistochemically demonstrated as described in the accompanying article [(3)].

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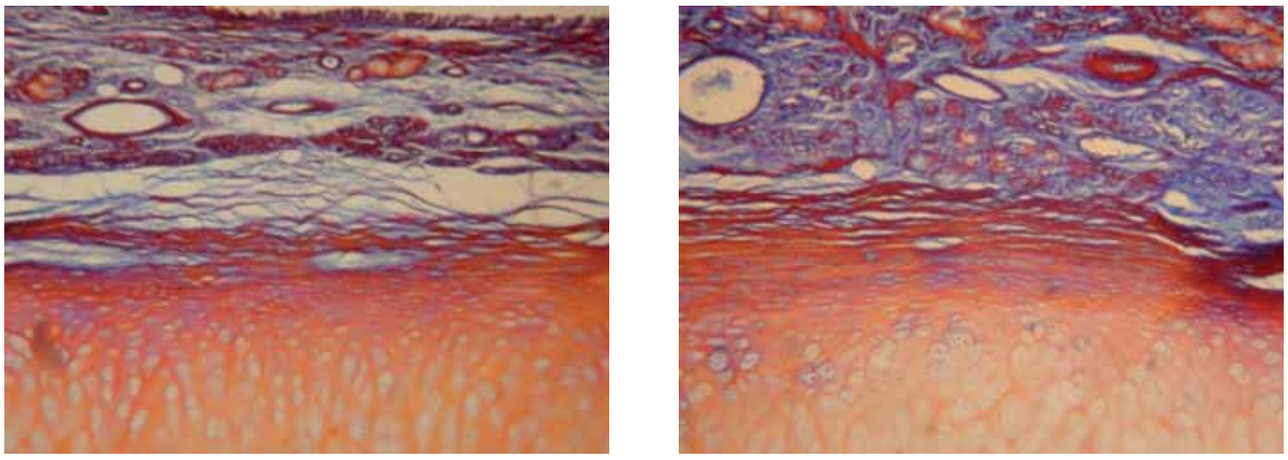

Figure 1. Septal perichondrium (Mallory-Cason staining). left) The existence of a dense inner and a loose outer layer is suggested but is probably due to a processing artifact. right) The perichondrium is a homogeneous structure without clearly distinguishable zones.

Table 1. Thickness (mean ± SD in μm) of the outer and inner perichondrium of the lateral crus of the right and left lobular cartilages and triangular cartilages.

	Lateral crus of lobular cartilage		**Triangular cartilage**	
	right side (n=13)	**left side (n=14)**	**right side (n=7)**	**left side (n=4)**
outer perichondrium	49.0 ± 11.1	47.5 ± 13.2	53.3 ± 10.4	46.5 ± 13.0
inner perichondrium	42.0 ± 10.4	37.9 ± 11.1	40.4 ± 17.3	36.0 ± 12.3

Measurements

In the Mallory-Cason stained sections, the thickness of the perichondrium of the triangular and the lateral crus of the lobular cartilages was measured with the aid of a graticule in the eyepiece of a microscope. Measurements were made on the inside and the outside of the cartilages. On every side of a cartilage, three parts of the perichondrium were measured at random. The mean values were used for statistical analysis. It was not possible to make truly random measurements on all sections. In case of artifacts, the part of the perichondrium closest to the artifact was measured. If there was any doubt about the validity of the measurements, the data were not included in the statistical analysis.

Statistics

The data were analyzed by paired *t* tests (one-tailed).

RESULTS

Septal perichondrium

Several of the Mallory-Cason stained sections suggest the existence of a dense inner layer and a loose outer layer in the septal perichondrium. In the same sections, however, parts of the perichondrium appear as a single layer of dense collagen bundles (Figure 1). In some specimens, the nose had not been processed as a whole unit but as individual septal and lobular cartilages instead. Especially in these specimens, it was difficult to distinguish between a dense inner and a loose outer layer. Therefore, the artifacts might suggest the existence of a loose outer layer.

The septal perichondrium is made up of a homogeneous dense layer containing collagen and elastic fibers (Figures 2 and 3). Its thickness is approximately 150-200 μm. The collagen is of type I (Figures 3 and 4). Type II collagen is not present in the perichondrium. The transition from cartilage to the perichondrium is not very well defined. Elastic fibers are not abundant, as demonstrated by Verhoeff-van Gieson stain (Figure 2).

Lobular and triangular cartilage perichondrium

The perichondrium of the lateral crus of the lobular cartilage consists of a homogeneous layer of collagen and elastic fibers (Figure 5). It is thinner than the septal perichondrium. However, in the lateral crus of the lobular cartilage, the outer perichondrium is distinctly thicker than the inner one. The perichondrium of the triangular cartilage gives a similar impression. Evidence was obtained by thickness measurements. The results demonstrate that the outer perichondrium

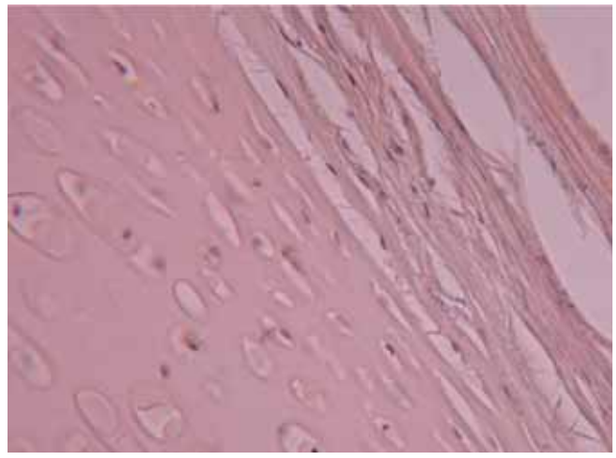

Figure 2. Septal perichondrium (Verhoeff-van Gieson staining). There are a few black-stained elastine fibers running from the perichondrium to the cartilage.

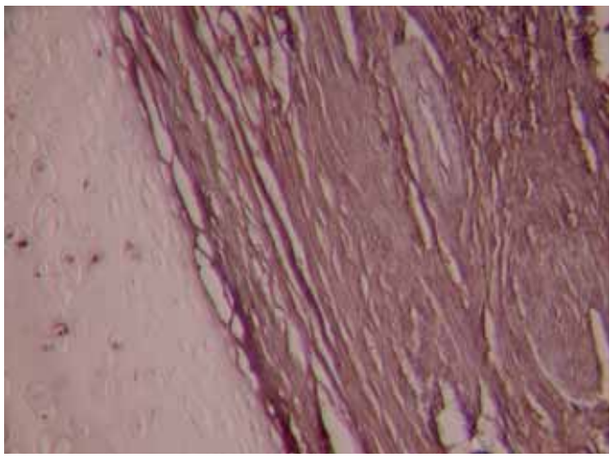

Figure 3. Septal perichondrium (type I collagen staining). There is an abundance of collagen type I in the perichondrium.

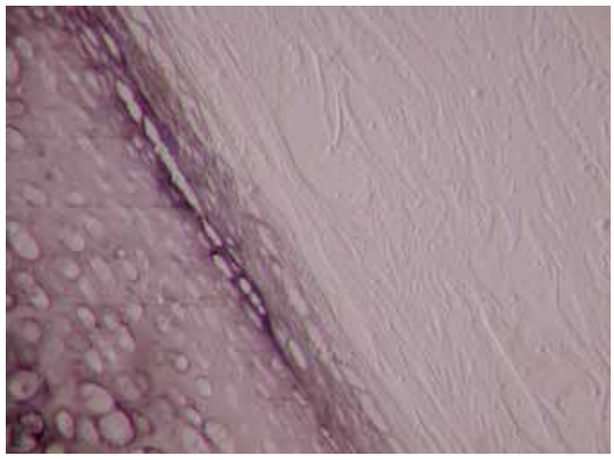

Figure 4. Septal perichondrium (type II collagen staining). There is no type II collagen in the perichondrium.

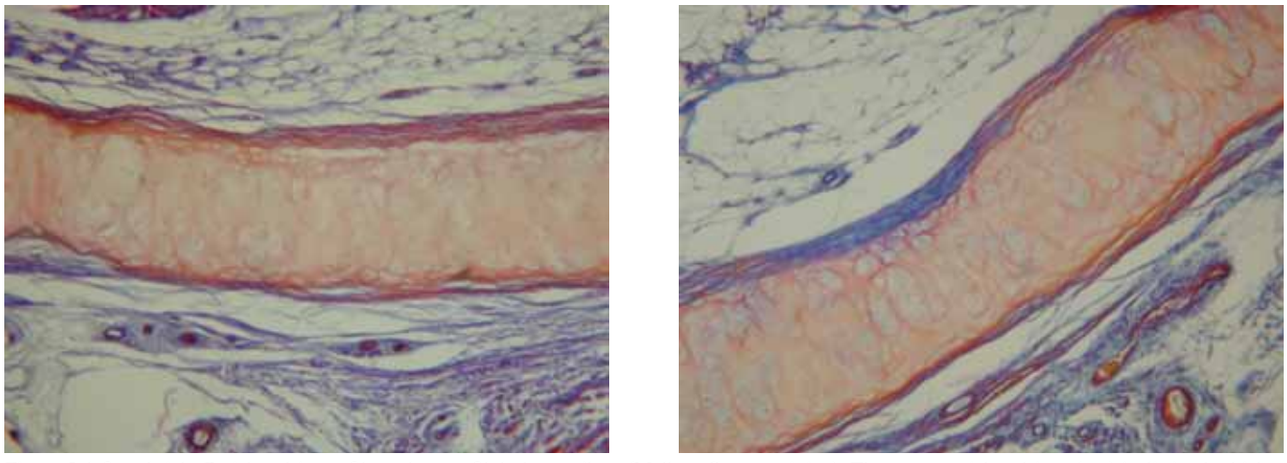

Figure 5. left and right Perichondrium of lateral crus of lobular cartilage (Mallory-Cason staining). The outer perichondrium (top) is distinctly thicker than the inner perichondrium.

is thicker than the inner perichondrium, both in the lateral crus of the lobular cartilage and the triangular cartilage (Table 1). The differences between the means are statistically significant for right and left lobular and triangular cartilages ($p < 0.05$). The differences between right and left sides are not statistically significant.

Both the Verhoeff-van Gieson and the Lawson staining of the perichondrium of the lobular cartilage demonstrate an abundance of elastic fibers (Figures 6 and 7). The number of elastic fibers clearly exceeds that of the septal cartilage. The numerous elastic fibers run either parallel to the surface of the cartilage or in oblique or perpendicular directions.

Immunohistochemistry demonstrates that the collagen is of type I and that type II is not present (Figures 8 and 9).

DISCUSSION

The main results of this histological study of the perichondrial envelope of the septal and lobular cartilages are the following: 1. the absence of clearly distinguishable zones in the perichondrium; 2. a significant difference in thickness between outer and inner perichondrium of the triangular and lateral crus of the lobular cartilage; and 3. a higher number of elastic fibers in the perichondrium of the lobular cartilage in comparison with that of the septal cartilage.

A major study by Bairati et al. on the structure of the perichondrium of various cartilages in animals and humans indicated the presence of three zones: 1. a loose outer layer containing blood vessels; 2. a main layer of dense connective tissue; and 3. an internal zone of fusion with the ECM of the underlying cartilage [(1)]. The authors included human nasal cartilages in their material, but the exact origin of these cartilages – lobular, triangular, or septal – is not clear. They concluded that in "nasal cartilages" the perichondrium is tightly attached to the underlying cartilage and that the border between the internal zone and the ECM of the cartilage is not very well defined, in contrast to ear cartilage. Our results are in line with this description; in many sections, it was hard to define the transition. This finding adds to the fact that in the nose the perichondrium shares a biomechanical role with the cartilage and that cartilage and perichondrium should be viewed as a functional unit. Our findings are not consistent with the sup-

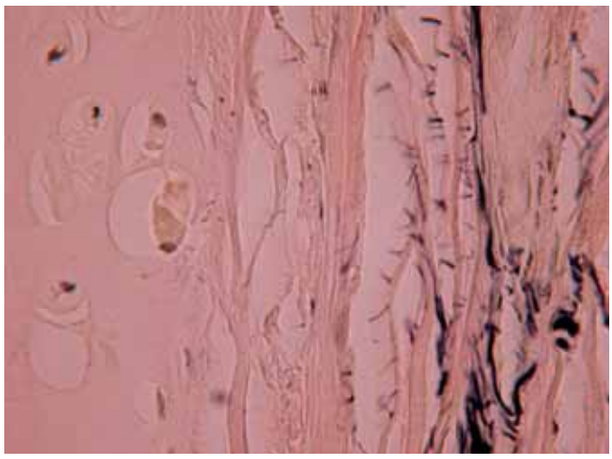

Figure 6. Perichondrium of lateral crus of lobular cartilage (Verhoeff-van Gieson staining). There is an abundance of black-stained elastine fibers that run parallel and perpendicular to the cartilaginous surface. Some fibers run in an oblique direction.

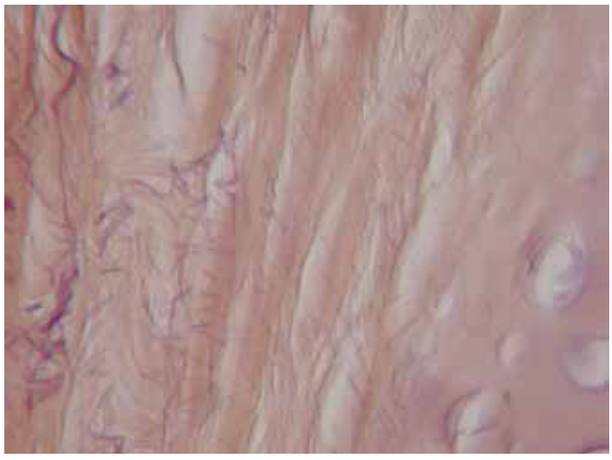

Figure 7. Perichondrium of lateral crus of lobular cartilage (Lawson staining). Many elastine fibers in the perichondrium run in various directions and form a network.

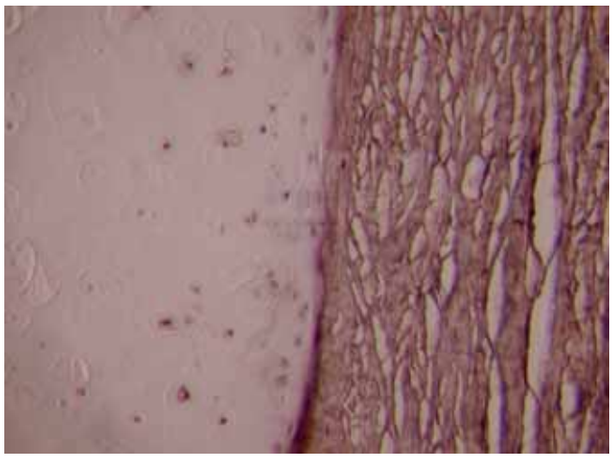

Figure 8. Perichondrium of lateral crus of lobular cartilage (type I collagen staining). There is an abundance of collagen type I in the perichondrium.

posed presence of a loose outer layer, however. In those sections where a loose outer layer was observed, it was highly likely that this was an artifact due to tissue fixation and sectioning. In many other sections, the perichondrium was seen as a homogeneous layer of collagen and elastic fibers. We therefore conclude that in both septal and lobular cartilages, the perichondrium does not have clearly distinguishable zones but is a homogeneous structure instead.

We speculate that differences in thickness of the outer and inner perichondrium in the lobular and triangular cartilages are related to the forces that are applied to the outer and inner sides of these cartilages. Muscles are found on the outside of the cartilages only. The dilatator naris muscle attaches to the lateral crus of the lobular cartilage. The transverse part of the nasalis muscle does not attach to the triangular cartilage but overlies it [(5)]. It moves the nasal skin; therefore, gliding movements between this muscle and the triangular cartilage should be permitted.

In our view, the finding of numerous elastic fibers in the perichondrium of the lateral crus of the lobular cartilage can be explained by its mobility. The posterior part of the lateral crus shows an in- and outward bending during respiration [(5)]. In contrast, the septal cartilage is more rigid, which is in line with its support function as discussed in the accompanying article [(3)]. It therefore needs fewer elastic fibers in its enveloping perichondrium. The direction of elastic fibers in the perichondrium is parallel to the surface of the cartilage but also oblique and perpendicular. This network-like arrangement helps maintain the complex shape of the lobular cartilage.

In conclusion, the outer and inner perichondrial envelopes of the septal, lobular, and triangular cartilages each have their own morphological characteristics that are related to functional differences between the cartilages.

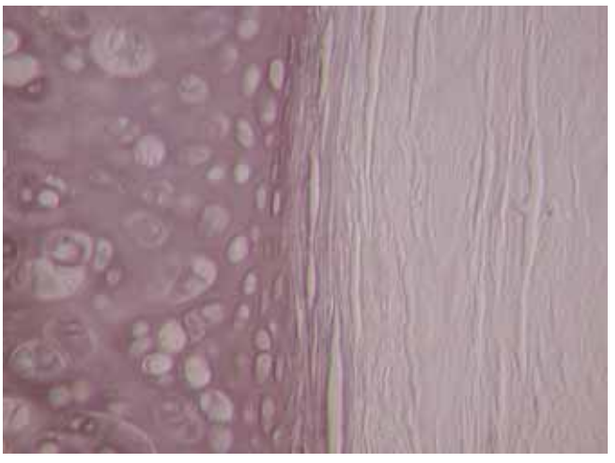

Figure 9. Perichondrium of lateral crus of lobular cartilage (type II collagen staining). There is no type II collagen in the perichondrium.

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Forty-one cases of congenital choanal atresia over 26 years - retrospective analysis of outcome and technique*

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SUMMARY

This retrospective analysis reflects the outcome of various techniques used in a series of 41 cases of choanal atresia treated at the Department of Otoloaryngology, Head- and Neck Surgery at the University of Mainz between 1980 and 2006. Thirteen bilateral and 28 unilateral cases are included. After endonasal management in 38 and a transpalatine approach in 3 cases a total of 15 patients needed revision surgery between 1 and 5 times to establish a stable result. Postoperative stenting was used in 23 patients with a failure rate of 35%, whereas only 11% of the 18 patients without stenting had to be revised. None of those 5 cases where Mitomycin C had been applied intraoperatively in combination with postoperative transnasal dilations needed surgical revision. We conclude that the endonasal micro-endoscopic surgical approach is successful if combined with postoperative dilations for up to one year. Stenting should be abandoned as it stimulates granulation formation that frequently leads to restenosis. The intraoperative application of Mitomycin C offers a promising adjunct in achieving a stable result.

Key words: choanal atresia,surgical technique, follow-up, stenting, mitomycin c

INTRODUCTION

In choanal atresia a widened vomer fuses with the narrow posterior nasal airway to an atretic plate, which can be either bony or membranous, resulting in an hourglass configuration to the nasopharynx and the choanal region. Among the reasons for failure after attempts of surgical correction in the past was sometimes inadequate analysis of the exact dimensions of anatomical components of the atretic plate but more frequently early restenosis of the choanal opening due to granulation and scarring. It is one of the most frequently observed congenital abnormalities of the nose with an incidence of 1 in 7000-8000 live births. Since its first description in 1755 its demographic characteristics have been repeatedly confirmed with a female predominance of 2:1 and a ratio of bilateral to unilateral of 40% to 60% [1,2]. The atretic plate is either purely bony (30%) or mixed of bony and membranous components (70%) [3]. Associations to other congenital abnormalities are present in about 50% and up to 75% in bilateral manifestations. The by far most frequent syndromal association in about 20% is the CHARGE – malformation. As suggested by Davenport et al. [4], two of the following defects must be present to establish this diagnosis: **C**oloboma, **H**eart, **C**hoanal **A**tresia, **R**etardation, **G**enito-urinary and **E**ar. Other isolated malformations frequently observed are meningoceles, hypertelorism and clefts. Different embryologic origins have been proposed, including a failure of the buccopharyngeal membrane to break and more recently another theory is the misdirection of mesoderm in the nasal cavities leading to a medial outgrowth of the horizontal and vertical process of the bony palate [5-7].

Bilateral atresia presents as a neonatal emergency as newborns are known to be obligate nasal breathers during the first 3-4 weeks of life. Immediately after birth the tongue is in contact with both soft and hard palate with the epiglottis being positioned above the soft palate. These infants therefore present with periodic respiratory distress and cyanosis, released by crying and pallor as well as severe feeding problems and aspiration. Immediate airway management and oropharyngeal airway intubation within the first hours of life are needed before planning surgical correction, which is performed within the first days of life. The diagnosis in most cases can be established by the inability to pass a catheter through either nostril into the pharynx and or endoscopic examination. However, today, state of the art workup includes a thorough endoscopic examination and a multi slice high resolution CT scan to analyze the individual anatomical topography as well as the bony or mixed bony / membranous nature of the atretic plate [3,8].

Over the last century four approaches have been discussed for surgical correction: Transseptal, transantral, transpalatine and trans- or endonasal, with the latter two still being frequently used. Since the advent of endonasal endoscopy and the

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endonasal microscopic technique, the endonasal approach has evolved to be the most common and is preferred today because of its excellent visualization and magnification that results in increased safety and reduced surgical time [9,10]. The use of various instruments has been described, including the use of bougies, dissectors, biting and cutting instruments, drills and lasers. To increase patency rates, some authors suggest the use of mucosal flaps to minimize the amount of corresponding raw surfaces while others have reported on the intraoperative application of Mitomycin C [8,11-13]. The most controversial aspect appears to be the use of stents for anywhere between 4 and 12 weeks [3,12]. However, stents have been associated with an overall failure rate of 30% regardless of the chosen approach or the respective surgical technique.

It is the objective of this retrospective analysis to discuss controversial aspects in the treatment of uni- or bilateral congenital choanal atresia and present a protocol based on the extensive experience gained from a large series of 41 consecutive cases at the Department of Otolaryngology, Head and Neck Surgery at the University of Mainz, Medical School in Germany.

MATERIAL AND METHODS

This is a retrospective, longitudinal and descriptive review of the surgical outcomes of a series of 41 patients with congenital choanal atresia. Of all 53 patients, who underwent surgery for uni- or bilateral choanal atresia between 1980 and 2006 at the Department of Otolaryngology, Head and Neck Surgery at the University of Mainz, Medical School in Germany, complete files, meeting the criteria for this retrospective analysis, were available in 41 cases. These inclusion criteria were: Cases of congenital choanal atresia with a complete chart available including medical notes on preoperative clinical course and symptomatology, documentation on diagnostic methods applied and clinical findings, operative reports on every surgical procedure, medical notes and / or reports on the postoperative course. Absence of symptoms or of endoscopic findings indicating restenosis had to be documented for at least one year postoperatively before the surgical procedure was assumed successful. However, in most cases more than one-year follow up was available, so long-term success could be evaluated. As documented in the patients' charts the individual follow up period varied between 14 months and 19 years. The mean follow up in our 41 patients was 41 months. All cases of acquired choanal obstruction or stenosis secondary to inflammatory or neoplastic disease, to trauma or to collagen disorders were excluded. Any respective files of our pediatric department or from other institutions involved with our patients were also included in the data acquisition. Such additional information was available in 31 of our cases. Of the 41 patients investigated 27 were female and 14 male. At the time of first surgical intervention the youngest girl was three days old and the oldest patient at the age of 67 years. Variables analyzed were: specifics of the individual malformation (bilateral, right – left), concomitant malformations, sex, age at first surgery, number of interventions necessary, initial symptoms, diagnostic procedures, surgical approach and surgical technique used, use of stents, use of Mitomycin C, surgical complications, postoperative choanal patency.

Statistics

Using SPSS 12.0 for windows, the complete database with all values for each variable was analyzed. Due to the small size of the studied patient group, the Fischer exact test was applied for statistical evaluation of the respective parameters and the relation between outcomes and surgical techniques

RESULTS

Thirteen patients had bilateral manifestations, 14 had unilateral disease on the right and 14 on the left side. There was no influence on the affected side by the respective gender of the patients. The most common syndromal manifestation was in our patient population was CHARGE association in 5 infants (12%), followed by Francescetti syndrome in 2 (5%), Fraley syndrome in 1, trisomia 4q in 1 and trisomia 22 in 1. In 5 of these infants with identified syndromal disorders and in 7 others, further isolated abnormalities were observed (Figure 1).

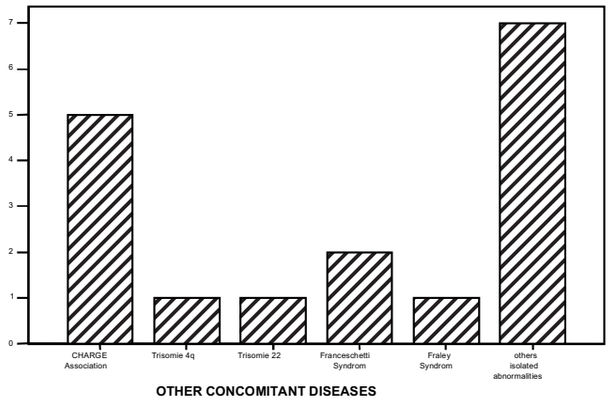

Figure 1. Incidence of concomitant diseases and deformities in 41 cases of uni- or bilateral congenital choanal atresia.

The most common diagnostic sign to establish the diagnosis was the inability to pass a small rubber catheter through the nose. This was the case in 28 patients, which included the 19 neonates, diagnosed in the delivery room. Nasal endoscopy was used diagnostically in 15 cases, a mirror test to ensure nasal breathing was diagnostically applied in 2 cases and one in 1984 in whom a plain radiograph with radiopaque medium was performed. Only 6 patients in this long-term review had a nasal CT scan for further evaluation. The classification of the nature of the atretic plate into bony or mixed bony / membranous was made based on the intraoperative findings. A purely bony plate was present in 15 cases (37%) while it revealed to have bony and membranous components were present in 26 cases (63%). The preferred approach for surgical correction of con-

genital atresia at present in the Department of Otolaryngology, Head and Neck Surgery in Mainz is the micro-endoscopic endonasal one. All, except 3, were operated endonasaly. The other three were operated on via a transpalatine approach due to an insufficient nasal passage. In a 40-year-old lady with unilateral atresia this was due to a severe endonasal deformity, which was corrected by an open septorhinoplasty at a later stage. In two secondary cases nasal passage challenged subsequent to complete restenosis by scarring and synechia formation. Between 1980 and 2006 this surgery was performed by 8 different senior otolaryngologists.

Looking at the number of surgical interventions necessary, 26 patients (63%) underwent only one procedure, 10 (24%) underwent two, 3 (7%) had three interventions while one needed four and another one five operations to achieve a satisfactory long-term result. The surgical instruments chosen depended on the nature and thickness of the atretic plate; these included

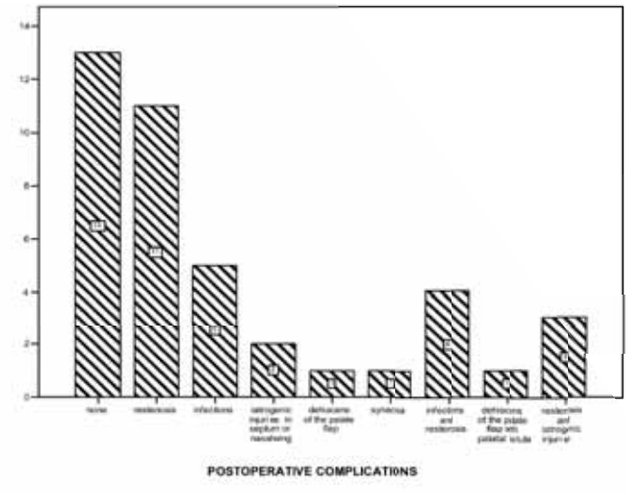

Figure 2. Frequency of various postoperative complications observed both short and long term.

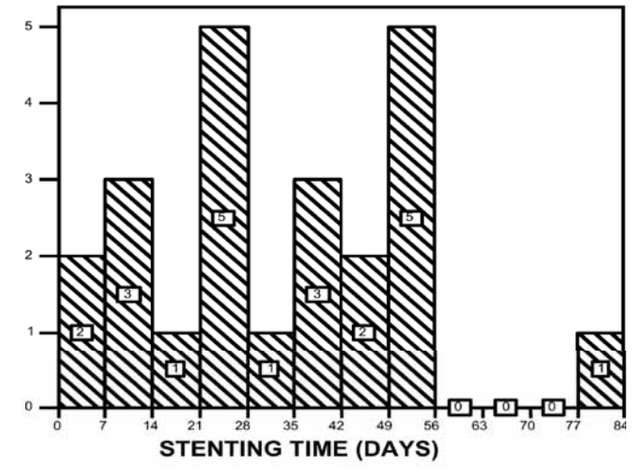

Figure 3. Analysis of the respective duration of postoperative stenting time in all individuals, in which stents were used.

Table 1. Comparison of surgical outcomes in patients treated with postoperative stenting as opposed to those treated without.

			surgical outcomes		
			success	**failure**	**Total**
Stents_vs._	Stents	Frequency	15	8	23
no stents		% of patients with stents	65%	35%	100%
	No Stents	Frequency	16	2	18
		% of patients without stens	89%	11%	100%
Total		subtotal	31	10	41
		Percentages	76%	24%	100%

bougies, dilators, cutting forceps and punches as well as powered drills, mostly used in combination. The drill was the most frequently used, serving as the most suitable tool to widen the medial aspect of the aperture by taking down the thickened vomer and posterior septum.

There were no life threatening intraoperative events such as spinal or skull base lacerations or severe bleedings. The postoperative complications observed are shown in figure two (Figure 2). To minimize the risk of the most common postoperative problem, which is restenosis of the created airway, stents were placed transnasally in 23 of our patients. The stenting time varied between 1 and 12 weeks (Figure 3). Comparing the rates of restenosis our data clearly reveal, that the group of the 23 stented patients had a failure rate of 35% compared to only 11% in the 18 patients where no stents were used (Table 1). The latter were followed by regular postoperative endoscopic examinations and repeated dilations with a soft rubber bougie. Dilations were performed in increasing intervals, performed by the surgeon initially on a daily basis. Starting after three weeks the number of dilations was reduced step by step to once a week and then performed by parents or patients themselves in a further outpatient follow up for up to one year. In five of the most recent cases we applied Mitomycin C intraoperatively (0.4 mg / ml for 10 minutes) as an additional effort to prevent restenosis. None of these individuals received stents. All were followed for more than 12 months so far and none had to be revised surgically.

DISCUSSION

The present retrospective analysis of the treatment of congenital choanal atresia over a period of 26 years, including 41 patients, constitutes one of the few reports on such a large number of cases in the literature [7,14-17]. The female/male ratio of 2 : 1 in this malformation [2,6,7,18] is in accordance with the literature. The right side predominance within the group of unilateral atresia, which was seen in other series [1,5,19] was not present in our patients, as we found both sides were equally affected with 14 manifestations. Brown et al. [3] reported a distribution of bony versus mixed bony and membranous atretic plates of 29% versus 71%, which is similar to our series. The timing of surgical intervention in bilateral cases is dictated by

the life threatening nature of the situation [18]. However, there is some debate on the most suitable timing for correction of unilateral choanal atresia. As there are few symptoms with the most common complaint being mucoid nasal discharge, sometimes accompanied by upper airway infections, headache and rhinolalia, some authors have suggested surgical repair when children are between 5 and 8 years old [6,20]. Stamm in contrast has performed unilateral correction in infants at the age of 6 months [8]. The benefit of an open airway and restored ventilation and drainage of the nasal and paranasal cavities have to be weighed against the potential need for revision due to relative narrowing as the created opening will not increase in size with growth of the child. In our series, over time there was a tendency towards earlier correction before school age, preferably around the age of two years. The embryologic etiology of congential choanal atresia has been attributed to a failure of resorption of the buccopharyngeal membrane and / or persistence of the nasobuccal membrane respectively in the past. After an evaluation of 37 cases by Hengerer et al. in 1982 [7] these theories appeared to have only little validity in a majority of cases. Hengerer instead hypothesized a misdirection of mesodermal flow secondary to genetic or environmental factors. The well described association of congenital choanal atresia with other malformations is indeed confirmed in our series with both syndromal and isolated deformities as has been detailed above. Besides CHARGE, Francescetti and Fraley syndromes we saw cases of trisomia 4q and a partial trisomia 22. However, the most common trisomia associated with choanal atresia in the literature is trisomia 21, known as Down´s syndrome, which none of our patients was diagnosed with. The partial trisomia 22 was attributed to a balanced translocation 11/22 in the mother. Complete trisomia 22 is the second most common reason for miscarriages after trisomia 16 and is rarely present in living neonates. However, the partial form is known to go along with a variety of pathologic physical findings with a cleft palate being among the most frequent. The male infant of our series with trisomia 4q presented with a right-sided choanal atresia. This genetic disorder is also rarely diagnosed and goes along with craniofacial disorders, malformation of the external ears and a prominent nasal bridge. Myers et al. [21] pointed out the potential role of maternal drug use, specifically of carbimazole. In our series however, we did not identify any clear pharmacological or environmental pathogenetic cofactors.

The majority in our series had the typical “delta deformity” of the widened vomer [16], which along with the medialized lateral walls of the posterior nasal cavity results in an hourglass shaped configuration of the choanal region and the nasopharynx. However, there was a broad variety of findings that varied with different craniofacial deformities, potentially associated with skull base defects. We would therefore, as a consequence of this retrospective analysis, strongly encourage any surgeon to whenever possible and available, achieve multiplanar CT scans with three-dimensional reconstructions to ensure adequate preoperative orientation and tailor the intervention to the individual pathology.

Our series reflects a clear preference for the endonasal approach, which has been widely described in the literature [19,22-24]. The excellent visualization allows for atraumatic and time effective repair using powered, miniaturized and endoscopic instrumentation. The combined micro- endoscopic endonasal approach has evolved as the best approach in our hands. The stereoscopic vision and magnification along with bimanual surgical manipulation offer a valuable adjunct to the use of the endoscopes, which combine excellent visualization with angled vision [10,25]. Figure 4 displays a case of right-sided bony atresia before (a) and immediately after (b) micro-endoscopic correction (Figure 4). Another option, especially in combination with this approach, is the use of CO2 lasers, which has also been applied in choanal atresia surgery [2,26]. The transpalatine approach was used in three of our patients

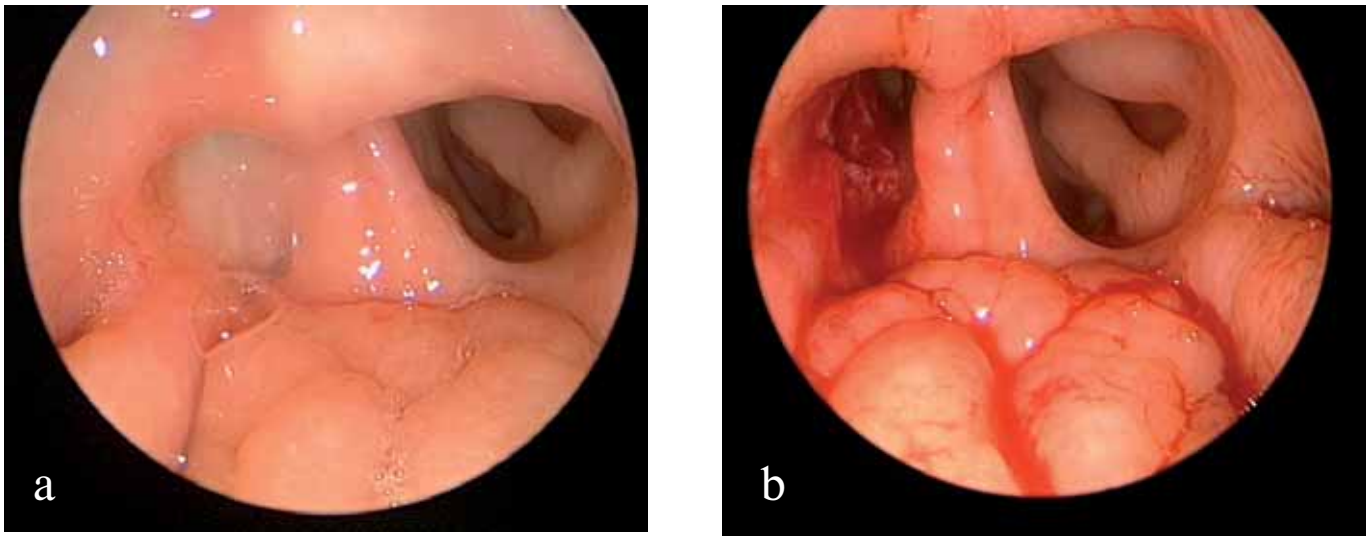

Figure 4. Endoscopic view of a combined bony-membranous atretic plate preoperative (a) and immediately postoperative (b).

and offers a good visualization in cases where the nasal passage is stenosed or obliterated to a degree that does not allow for endonasal instrumentation. However, a marked drawback of this procedure is the known long-term complication rate, resulting from palatal muscle dysfunction and consecutive mandibular joint pathology as well as orthodontic problems in 50% of the cases [1,18,19]. As a consequence, we would reserve this technique for cases of insufficient endonasal visualization in revisions or children older than 5 years.

Any outcome analysis of congenital choanal atresia centers on intra- and postoperative measures to ensure a stable result over time. Some authors propose mucoperiosteal flaps to be created around the created opening by asymmetric incisions or in a cross over technique in an effort to minimize or eliminate corresponding raw surfaces [8,11,12]. From the data of our series we cannot evaluate the effectiveness of these techniques. They have not become part of our routine due to rather inhomogeneous anatomical situations and the difficulty of respective adequate postoperative control in neonates and small children. A clear difference was seen between our patients with and without postoperative stents with an incidence of restenosis of 35% and 11% respectively. Difficulties related to stenting in repair of choanal atresia have been discussed in the literature mostly as a consequence of the circumferential pressure, leading to ischemia, osteoblastic and fibroblastic reactions and a lack of reepithelization in the choanal aperture as well as pressure related lesions of the collumella, alar cartilages and more posterior parts of the septal cartilage [6,12,22]. Granulation tissue has been associated with a 30% failure rate with the use of stents regardless of the surgical technique or approach [27]. As a consequence of our own experience and in accordance with the cited publications of other authors we have abandoned the use of stents.

As a more recent change in treatment modalities used in our series, we have applied topical Mitomycin C intraoperatively in the last 5 cases, combined with repeated postoperative dilations. After sufficient long term follow up of over at least 1 year, we did not have to revise any of these 5 patients. There is plenty of evidence from the literature on the efficacy of topical Mitomycin C in reducing scar formation and restenosis, especially in the aerodigesitve tract [28]. The cytostatic effects of this antibiotic substance and its inhibitory effects on fibroblast proliferation have, in accordance with our data, already been described as helpful in choanal atresia repair [13,29].

Taking all information gained from this series of 41 patients into account and relating it to the recent literature we are now using the following protocol for the treatment of congenital choanal atresia:

- Subsequent to initial airway management, bilateral choanal atresia is corrected within the first week of life. In cases of unilateral atresia surgical correction should be electively performed before school age, preferably around the age of 2 years.
- If the nasal airway is not severely compromised by concomitant malformations and deformities, the micro- endoscopic, endonasal approach is preferable and offers numerous technical options and variations.
- The atretic plate is to be removed with all compounds, based on preoperative analysis of the individual deformity and knowledge of potential concomitant malformations with the help of multiplanar high resolution CT scans and 3D reconstructions.
- Topical Mitomycin C is applied intraoperatively for 10 minutes in a dilution of 0.4 mg / ml to reduce excessive granulation and scar formation.
- In the postoperative management, repeated endoscopic controls are combined with transnasal dilations with a soft rubber bougie in increasing intervals, performed by the surgeon initially and by parents or patients in a further outpatient follow up for up to one year.
- No stents are applied into the nasal airways.

CONCLUSION

In conclusion, this thourough analysis of a series of patients presents a protocol for the treatment of congenital choanal atresia. The endonasal micro-endoscopic surgical approach is successful if combined with postoperative dilations for up to one year. Stenting should be abandoned due to immanent granulation formation frequently leading to restenosis. The intraoperative application of Mitomycin C offers a promising adjunct and may play a role in achieving stable long-term results.

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Screening of olfactory function using odourized markers*

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SUMMARY

Background: *The goal of our study was to create a psychophysical test for the screening of olfactory function on the basis of commercially available odourized markers (OM). There are six coloured markers in one package filled with different odourants at suprathreshold levels. In order to identify the best approach, we investigated five different variations of the technique.*

Materials and methods: *Olfaction was investigated in 189 subjects. Healthy participants as well as patients suffering from olfactory disorders were tested. Initially subjects were tested by one of five methods using OM. Finally, the "Sniffin' Sticks" test (butanol odour threshold, odour identification) was performed.*

Results: *Correlation of the OM screening test and the "Sniffin' Sticks" ranged from 0.49 to 0.93 indicating that variations of the technique strongly influence the results of testing. The best technique for evaluating olfactory function included spontaneous naming of odours and odour identification from a list of four distractors. The sensitivity of this method was sufficient to determine anosmia.*

Conclusions: *The odourized markers screening test can be used to screen for anosmia in the general population. However, the precise quantification of olfactory function is not possible, because of the relatively small amount of odours.*

key worlds: odourized markers, Sniffin' Sticks, olfaction, screening test

INTRODUCTION

The sense of smell and its disorders are frequently neglected by physicians. Although many tests of subjective olfactometry have been introduced, it is a particular challenge to find a valid olfactory test that is both affordable and readily available.

Impaired olfactory function decreases the quality of life and can lead to life threatening and hazardous events (gas-poisoning, cooking-related incidents or ingestion of spoiled food) [(1)]. Early diagnosis of olfactory disturbances can initiate preventive precautions. For example, olfactory screening could help identify individuals at high risk of being exposed to toxic substances, because their inability to smell poses a hazard. This is particularly important for employees in certain jobs, such as workers in chemical factories, who can be exposed to high doses of volatile, toxic chemicals.

Assessment of the olfactory function prior to nasal surgery is important from the medico-legal point of view [(2)].

Nasal obstruction and change of smell is the most common complaint of patients suffering from sinonasal disease [(3)]. Olfactory dysfunction can be a symptom of neurological disorders. Olfactory tests can help to distinguish Alzheimer's disease from other types of dementia [(4)] and the diagnosis of Parkinson's syndrome can be supported by olfactometry as well [(5)]. Thus, inexpensive olfactory tests would be useful.

To identify the most reasonably priced standardized test to assess olfactory function, we decided to use odourized markers (Figure 1), which are originally designed for use by children. We chose a package that included 6 coloured and odourized markers (Centropen®a.s., Art. 2589/6 Perfumes, Dacice, Czech Republic), each with a different colour, and each having a unique odour. The purpose of this study was to establish a simple, short and valid technique for olfactory screening on the basis of OM.

MATERIALS AND METHODS

The study was performed according to the Declaration of Helsinki (Summerset West amendment) on guidelines for biomedical research involving human subjects. It was approved by

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the Ethics Committee of the Regional Hospital Pardubice. All subjects provided written consent after they were thoroughly acquainted with all details of the investigation.

Figure 1. Commercially available odourized markers.

Participants

Olfactory functions were assessed in 189 subjects. Mean age of participants was 47.4 years, standard deviation 16.8 (age range 16 – 83 years); 115 men and 74 women took a part in our study. Healthy people as well as patients suffering from olfactory disorders were tested. We included patients and staff of the Department of Otorhinolaryngology & Head and Neck Surgery of the Regional Hospital Pardubice. Participants assessed their sense of smell as normal in 105 cases, decreased in 43 cases, completely impaired in 36 cases, and altered in 5 cases. A total of 74 people did not suffer from any disease (15 of whom were smokers) that could influence olfaction and were verified as to having normal olfactory function. A total of 68 patients suffered from sinonasal disease. Head trauma, upper respiratory tract infection, and idiopathic etiology were presented in 12, 14, and 4 patients, respectively. Seventeen patients suffered from diseases that could influence olfaction (psychiatric diseases, neurodegenerative diseases, tracheotomized patients and people exposed to toxic substances).

Study design

Initially subjects were tested with one of five techniques using odourized markers (OM). Finally, the "Sniffin´ Sticks" test (butanol odour threshold, odour identification) was performed. In addition, the subjects' history was taken and nasal endoscopy was performed. For evaluation of each technique, we included both normosmic subjects and subjects with olfactory loss as ascertained by means of the "Sniffin' Sticks".

Olfactory testing

Subjects were first tested with the OM set, then with "Sniffin' Sticks". Testing was performed in a quiet room with adequate ventilation.

The OM screening test includes 6 coloured and odourized pens. The black pen smells like liquorice, the yellow pen like lemon, brown like cinnamon, blue like raspberry, green like apple, and the red pen smells like strawberry. The exact chemical composition of the cartridges of pens is the trade secret of the producer. Pens are filled with water-soluble pigments and aromas. The OM are designed for children from 3 years of age and are non-toxic. The product matches the requirements of European Norm (EN-71) used for safety of toys. The producer guarantees the quality for at least of 2 years. The odours of the markers are of the same intensity when properly used, meaning that the top of the pen has to be covered properly after each use. There is standard filling of the markers in the factory with a quality control process in their manufacturing. A random control of the final product is performed.

In order to test odour identification, forced choice technique was used. The five techniques employed differed in the following aspects: (1) presented distractors were different, (2) repeated naming of odours was added to one of the techniques, (3) spontaneous naming was used in two techniques. When olfaction was tested with the OM set, subjects were blindfolded to prevent visual identification in four techniques. Colours of markers were uncovered only in Experiment B meaning that in this experiment subjects knew the colour of the odourized markers.

Description of techniques used

Based on results of three Preliminary experiments, we created 2 new techniques of testing olfaction and decided to validate them in a larger number of subjects. In the following, we will first describe the 3 Preliminary experiments preceding Experiments A and B.

Preliminary experiment 1: The black marker was presented first for 4 s and subjects were asked to select the appropriate descriptor from a list of four distractors (in this case, it was "liquorice", "raspberry", "paprika" and "hospital"). Then, they were asked to select another probable distractor. Thus, subjects selected two descriptors and labeled them as more appropriate or less appropriate. If subjects selected the correct answer (e.g., "liquorice") they received 2 points. If subjects had a "near miss" (e.g., "hospital"), they received another point (see Table 1 for descriptors and scoring).

Table 1. List of distractors of Preliminary experiment 1. Correct answers are shown in boldface (2 points), "near misses" are italic (1 point).

liquorice	raspberry	paprika	*hospital*
glue	**lemon**	leather	*perfume*
paprika	*clove*	**cinnamon**	coffee
mushroom	garlic	*strawberry*	**raspberry**
deodorant	**apple**	spice	meat
mint	**strawberry**	tomato	thinner

A maximum of 3 points for one odour presentation was possible. The same method was used for the yellow, brown, blue, and green markers. Because of the very artificial odour of the red marker, only one appropriate descriptor had to be selected. For the correct answer subjects received 2 points. The intention was to minimize the influence of the poorly identifiable odour of the red marker. The minimum and maximum score from this test was 0 and 17 points respectively.

Preliminary experiment 2: The principle of the second technique remained the same. We changed some of the incorrect descriptors (e.g. "paprika" to "cigarette" for the brown marker) and the correct descriptors as well ("strawberry" to "soap" for the red marker). We predicted greater differentiation between hyposmia and normosmia based on described changes than the results indicated.
The selection of two distractors (proper and "near miss") in Preliminary experiment 1 and 2 was not always understood by the subjects and the explanation of the technique prolonged the testing time. Therefore we decided to change the technique of testing.

Preliminary experiment 3: Spontaneous naming was added to the third technique. People were asked to name the odours first. Each of the markers had to be described by a different name. Subjects got one point for naming each of the odours differently (in total 6 points). Then the list of 4 distractors for every colour was presented. Some of the incorrect distractors were changed. The subjects had to choose only one descriptor. When the identification of the odour was correct, participants received 2 points, if subjects had a "near miss", they gained only 1 point. The purpose of this preliminary experiment was to eliminate the artificial character of the odours by enabling subjects to name the odours based on their own experiences. Descriptors were changed based on experiences with previous techniques.

Experiment A: Repetitive naming of odours was added to this definitive technique. People were asked to identify the odour from a list of 4 distractors for black, consecutively for yellow and brown marker. The list of distractors was the same as in Preliminary experiment 3. Subjects could choose only one distractor. If the answer was right, they gained 2 points for each odour. If the answer was "near miss", they gained 1 point. The same markers were presented again (black, yellow, brown), but in a different, randomized sequence. Subjects were asked to identify and name the odours again based on their previous identification from list of distractors. They gained another point, if the answer was right. The same procedure was done with the blue, green and red colours. For identifying red colour from 4 distractors subjects gained only one point to minimize the influence of this very artificial odour. The minimum and maximum score was 0 and 17 points respectively. The purpose of this technique was to increase the amount of smell stimulus by repetitive presentation of the odours.

Experiment B: This definitive technique was based on spontaneous naming and odour identification from the list of four distractors. People were asked to name the odours first. Each of the markers had to be described by different names. Subjects scored one point for naming each of the odours differently. If they could not name the odour or gave the same name to the odours, they scored 0 point. Then subjects had to choose one correct answer from the list of four distractors. The list was changed radically in order to respect the colours of the markers (Table 2). For example, when the yellow marker was presented, distractors of yellow colours were offered ("banana", "lemon", "apple" and "pineapple"). Subjects gained one point for the correct identification. The minimum and maximum score was 0 and 12 points respectively.

Table 2. List of distractors (Experiment B). The correct answers are boldfaced.

liquorice	pepper	paprika	currant
banana	**lemon**	apple	pineapple
chocolate	tea	**cinnamon**	coffee
grapefruit	strawberry	orange	**raspberry**
paprika	**apple**	kiwi	banana
orange	mandarin	**strawberry**	currant

"Sniffin' Sticks"
The comparison of olfactory function was performed by "Sniffin' Sticks" (threshold and odour identification), which is based on pen-like odour dispensing devices [6]. Odour discrimination, which is a part of "Sniffin' Sticks" test, was left out due to time constraints. Odour thresholds were determined using n-butanol as the odourant. For odour identification, 16 odourants were presented to each subject. In order to identify the odourant, a list of 4 descriptors was presented. The exact technique of testing is described by Hummel et al. [6].
When subjects scored less than 9 of 16 points in odour identification and were not able to detect n-butanol in its highest concentration in threshold testing, the subject was determined to be functionally anosmic.

Nasal endoscopy
After olfactory tests were completed, nasal endoscopy (Karl Storz, Hopkins Optic, 30°, 2.7mm, 11cm) was performed to assess possible pathology in the nasal cavity and nasopharynx.

Statistical analysis
General participant data from each experiment are presented in Table 3. Data were further investigated using SPSS 12.0 for Windows®. Correlation coefficients of threshold, identification, and OM test were calculated. Correlations were performed between scores from the various tests and age of each subject (Table 4).
For Experiment A and B t-tests were used to compare data of threshold, identification and OM screening test between

patients with sinonasal and posttraumatic olfactory loss, and for nasal endoscopicy. ROC (Receiver/Operator Characteristics) analysis was done to evaluate sensitivity and specificity for both techniques (Table 5).

Table 3. Number of subjects, number of patients with functional anosmia stated by "Sniffin' Sticks", mean age and standard deviation, and sex of participants in each experiment.

experiments	experiments	number of anosmics	mean age of participants ± standard deviation	sex of participants male/ female
Preliminary 1	22	4	46.5 ± 15.9	9 / 13
Preliminary 2	17	3	51.8 ± 17.7	15 / 2
Preliminary 3	25	7	45.1 ± 18.1	17 / 8
Experiment A	54	12	46.8 ± 16.2	37 / 17
Experiment B	71	7	47.9 ± 16.6	37 / 34
total	189	33	47.4 ± 16.8	115 / 74

Table 4. Correlation coefficients of all techniques with "Sniffin' Sticks" (butanol odour threshold and odour identification) and age.

experiments	threshold	identification	age
Preliminary 1	0.746	0.927	-0.433
Preliminary 2	0.546	0.493	-0.196
Preliminary 3	0.870	0.856	0.001
Experiment A	0.736	0.648	-0.527
Experiment B	0.707	0.747	-0.158
age	-0.247	-0.270	1.000

Table 5. Sensitivity and specificity of Experiment A and B of OM test for functional anosmia stated by "Sniffin' Sticks".

Eperiment A			Eperiment B		
cutoff value	sensitivity	specificity	cutoff value	sensitivity	specificity
2	0.08	1.00	0	0.14	1.00
3	0.17	1.00	1	0.43	1.00
4	0.42	0.98	2	0.43	0.98
5	0.58	0.98	3	0.57	0.97
7	0.67	0.98	4	0.86	0.95
8	0.83	0.93	5	1.00	0.94
9	0.83	0.83	6	1.00	0.91
10	0.92	0.74	7	1.00	0.81
11	0.92	0.62	8	1.00	0.67
12	0.92	0.50	9	1.00	0.56
13	1.00	0.33	10	1.00	0.36
14	1.00	0.24	11	1.00	0.19
15	1.00	0.10	12	1.00	0.00
16	1.00	0.00	-	-	-

RESULTS

Analyses of results from Preliminary experiment 1, 3, Experiment A and B indicated significant correlation between results from these techniques and testing with the "Sniffin' Sticks" test battery ($p<0.01$). Correlation coefficients of all techniques are presented in Table 4. A negative correlation was found in all techniques, except Preliminary experiment 3, between scores from the OM tests and age, which was in line with the results from the "Sniffin' Sticks".

Preliminary experiments were used to create the final method of olfactometry using odourized markers. Therefore, we present only the final results of Experiment A and B.

Subjects suffering from sinonasal diseases achieved lower scores in both Experiments when compared to healthy subjects ($p<0.01$). The difference between scores from patients with posttraumatic olfactory loss and healthy controls was also significant for OM test in both Experiments ($p<0.01$).

Nasal endoscopy revealed polyps in 15 subjects of both Experiments. Results of olfactory tests (Experiments, butanol odour threshold and odour identification) of subjects with polyps were significantly decreased in Experiment B ($p<0.05$), but only with regard to threshold testing in Experiment A (p values of identification and OM test were 0.07 and 0.32, respectively). Sensitivity and specificity of the technique of Experiment A for the diagnosis of anosmia were 100% and 33% and for Experiment B 100% and 94%, respectively (ROC analysis) (Table 5). These results suggest the second technique as acceptable for the screening of olfactory function.

DISCUSSION

Many olfactory tests have been established. "Sniffin' Sticks" and UPSIT (University of Pennsylvania Smell Identification Test) are among the most commonly used tests in Germany and the USA, respectively. Both tests provide valid information on olfactory function. "Sniffin' Sticks" test enables in its extended version threshold testing using n-butanol. UPSIT is based only on suprathreshold testing using 40 odours. It is not possible to perform a threshold measurement with any number of suprathreshold stimuli. To satisfy the need for a shorter screening test, both methods have their own shorter variations. Hummel et al. described a method using 12 common odours to distinguish severe olfactory dysfunction from normal olfactory function [7]. The test has advantages in terms of costs, because it can be used repeatedly for approximately 1 year. Shorter and faster variations of UPSIT are also available. Doty et al. described the development of the Cross-Cultural Smell Identification Test (CC-SIT) [8], which includes 12 odours and can be administered in less than 5 minutes. Advantages of this test are self-administration technique and incorporation of "multicultural" odourants. In addition, 3-item smell identification test Q-SIT (Quick Smell Identification Test) has been reported to screen for patients with anosmia. On the other hand, the specificity of the Q-SIT for anosmia was only 40%. There are other reports on the screening test of olfaction [9-10].

The present study investigated the application of odourized markers in the screening of olfactory function. Only suprathreshold testing is possible using OM. We decided to compare our test with the "Sniffin' Sticks". This decision was based on 4 reasons: (1) the technique of pen-like odour dispensing devices is similar, (2) odours in daily life of Germans and Czechs do not differ much, (3) threshold and suprathreshold testing is possible using "Sniffin´ Sticks" test and (4) the

test is reliable in detecting anosmia [6].

The final technique (Experiment B) of testing smell ability using OM was developed on the basis of several preliminary experiments. Negative correlation of OM screening test scores with age and the significant correlation to "Sniffin´ Sticks" test results (threshold and odour identification) validated its usefulness for olfactory screening. Additionally, this conclusion is supported by significantly decreased scores on an OM screening test in subjects suffering from sinonasal and posttraumatic olfactory loss. Good sensitivity (100%) and satisfactory specificity (94%) were achieved with regard to detecting anosmia.

Advantages of the present test are: low cost (approximately € 1,- per set), the possibility of repetitive use of one test, and its availability in regular shops. Among the major disadvantages of the OM screening test are: small numbers of tested items, the colours of the markers, and the artificial character of odours. Despite these facts, we conclude that OM screening test (presented in Experiment B) can be used for orientation assessment of olfactory function and as a screening method of anosmia in the general population.

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Histamine induces nasal obstruction via calcitonin gene-related peptide in sensitized guinea pigs*

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SUMMARY

The purpose of this study was to characterize the late phase nasal obstruction that is induced by a nasal histamine challenge in sensitized guinea pigs. The volume of the nasal cavity was measured using an acoustic rhinometer. A nasal histamine challenge to unsensitized animals induced nasal obstruction at 30 minutes after the challenge while a challenge to sensitized animals induced nasal obstruction not only at 30 minutes but also at 4-6 hours. Histamine (measured by high-performance liquid chromatography), cysteinyl leukotriene (enzyme-linked immunosorbent assay (ELISA)), prostaglandin D_2 (ELISA), eosinophils and basophilic cells of sensitized guinea pigs were not changed in the late phase after histamine challenge. Administration of pyrilamine, a histamine H_1 receptor antagonist, and calcitonin gene-related peptide (CGRP) (8-37), a CGRP-1 receptor antagonist, significantly improved histamine-induced nasal obstruction at 30 minutes and in the late phase, respectively. These results suggest that a nasal histamine challenge induces nasal obstruction not only immediately through the histamine H_1 receptors but also in a late phase via CGRP.

Key words: histamine, CGRP, nasal obstruction, acoustic rhinometer, guinea pig

INTRODUCTION

Allergic rhinitis is one of the most prevalent allergic disorders, with symptoms of sneezing, nasal discharge and nasal obstruction [(1)]. Histamine [(2)] plays a very important role in the nasal obstruction of nasal allergy patients. Histamine not only induces symptoms of rhinitis directly, but also enhances infiltration of eosinophils [(3)]. Histamine enhances the expression of intercellular adhesion molecule-1 (ICAM-1) on human vascular endothelial cells [(4)], and the production of superoxide anion from eosinophils [(5)]. Histamine also affects the T helper type 1/T helper type 2 (Th1/Th2) balance [(6)]. The effects by histamine vary according to the state of sensitization of the host [(7)]. Histamine-induced eosinophil infiltration appears to be more remarkable in the allergic than the non-allergic state [(8)]. The increased reactivity in the sensitized state is called "hypersensitivity", in which allergic responses are induced by lower doses of chemical mediators than those needed to evoke responses in non-allergic states [(9)].

In a guinea pig model of nasal allergy, although mediators including cysteinyl leukotrienes (CysLTs) [(10)], platelet activating factor (PAF) [(11)], nitric oxide [(12)], and neuropeptides [(13)] have been suggested to be associated with the nasal obstruction, histamine is the most important chemical mediator [(14)]. Histamine induces rhinitis symptoms directly [(15)]. Histamine stimulates vascular permeability, glandular secretion, production of superoxide anion from eosinophils [(5)], and sensory nerves, and release of both calcitonin gene-related peptide (CGRP) and substance P from the terminals of the trigeminal nerve [(15, 16)]. As in humans, the effects of histamine vary between sensitized and unsensitized animals [(17)]. The degree of infiltration of mast cells, which contain large amounts of histamine, in the nasal mucosa is also different between the allergic model and the normal state [(18)].

Thus, although histamine has an important place in early phase nasal obstruction in allergic rhinitis [(2)], there is a possibility that histamine plays an important role in late phase nasal

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obstruction also. However, no information about nasal obstruction by histamine in the late phase has been reported. Therefore, in this study, we compared the nasal obstruction induced by a nasal histamine challenge in both sensitized and unsensitized guinea pigs using an acoustic rhinometer (GJ Elektronic, Skanderborg, Denmark). The acoustic rhinometer can measure nasal obstruction noninvasively using acoustic reflections and has been widely used in clinical diagnosis [19]. The acoustic rhinometer modified for application to guinea pigs has been developed and found to be a precise and useful method for evaluating nasal obstruction in experimental allergy model animals [20]. Furthermore, we analyzed the infiltration of inflammatory cells into nasal mucosa, concentrations of chemical mediators in intranasal perfusate, and the effect of mediator receptor antagonists on histamine-induced nasal obstruction to elucidate the mechanism of pathogenesis.

MATERIALS AND METHODS

Drugs

Histamine dihydrochloride, pyrilamine maleate salt, atropine sulfate salt hydrate, N-omega-nitro-L-arginine methylester (L-NAME), N-omega-nitro-D-arginine methylester (D-NAME), CGRP (8-37) and urethane were purchased from Sigma (St. Louis, MO, USA). Other reagents used were dinitrophenilated Ascaris suum (DNP-As; LSL, Tokyo, Japan, Lot: 747042) and aluminum hydroxide ($Al(OH)_3$; Wako Pure Chemicals, Osaka, Japan). Water was used to prepare the pyrilamine the others were prepared by saline.

Animals

Male Hartley guinea pigs were purchased from SLC Inc. (Shizuoka, Japan) and housed at constant temperature (23 ± 2˚C) and humidity (55 ± 10 %). All experiments were conducted according to the institution's guidelines for care and use of laboratory animals in research.

Sensitization and histamine challenge of guinea pigs

Sensitization of guinea pigs was performed according to the method of Ishida et al. with a slight modification [21]. Specifically, guinea pigs were actively sensitized with antigen using an intraperitoneal (i.p.) injection of 10 μg dinitrophenilated Ascaris suum (DNP-As) containing 1 mg $Al(OH)_3$ in 1 mL saline. A booster i.p. injection of the same concentration of a DNP-As / $Al(OH)_3$ mixture in 1 mL of saline was given per animal at 2, 4 and 6 weeks after the first sensitization. Two weeks after the last i.p. injection, 0.005 % DNP-As saline was intranasally administered by a spray twice a day, at a dose of 0.5 mL per day, for 10 days. The sensitized animals were used within 7 days after the end of the sensitization.

For histamine challenge, each nostril of both sensitized and unsensitized guinea pigs was instilled with either 20 μL of 0.1 mg/mL histamine. Saline was used as a control challenge.

Measurement of nasal cavity volume

Measurement of nasal cavity volume was performed by the method of Nakamoto et al. with a slight modification (14). Specifically, the nasal cavity volume in guinea pigs was measured using the acoustic rhinometer after anesthesia by urethane (1.0 mg/kg, i.p.). The "pre" time point represented acoustic rhinometry that was performed 15 minutes before either histamine or saline intranasal challenge. Acoustic rhinometry was performed 10, 30 and 60 minutes, and then every hour after the intranasal challenge for an 8-hour period.

In acoustic measurements, a generated sound impulse is passed into one nostril, and the reflection from the nose is captured by a microphone. Computerized analysis (Nadap v2.31c) of intensity and time delay from the nasal cavity was used to determine the nasal cavity volume. Nasal cavity volumes were estimated from the nostril to 2 cm into the nasal cavity. The sum of right and left values of nasal cavity volume were analyzed. Changes in volume after the intranasal challenge were expressed as the percentage change from the "pre" values. For each animal, the mean value of consecutive 3 measurements for each nostril was calculated. This measurement was repeated 3 times in each nostril and the median of the 3 mean values was used as the volume at that time point. In the experiments using animals, the cavity volume has been known to be a reliable parameter. An inverse correlation between individual percent change of nasal resistance and nasal cavity volume was statistically significant [14]. The intranasal instillation of histamine causes a dose-dependent reduction in percent change of nasal cavity volume in the challenged side [14].

Intranasal perfusion

After nasal instillation of either histamine or placebo, animals were anesthetized and placed in a supine position. The trachea was transected into two sections, one of which continued to have spontaneous respiration and the other had a cannulated polyethylene tube filled with saline attached to the nares. The tube was connected to a perfusion pump (Tubing Pump Rotor, Type 25N, Taitec, Saitama, Japan) for intranasal perfusion of saline at a rate of 0.2 mL/minute. Six hours after the histamine or saline challenge, 10-minute intranasal perfusates were done in each animal. The perfusates were collected in a container cooled by ice water and divided into 3 aliquots for measurement of histamine, CysLTs and prostaglandin (PG) D_2.

In some sensitized animals, repeated intranasal perfusates were performed for 10 minutes at 3 and 5 hours after the nasal histamine challenge.

Measurement of histamine

Histamine in the intranasal perfusate was quantified as described previously [22]. Briefly, 0.5 mL of each perfusate was mixed with 50μL of 30 % perchloric acid (Wako Pure Chemical) and kept at -30˚C overnight. The sample was centrifuged at 8,000 g for 15 minutes at 4˚C. The fluorescence method using a high-performance liquid chromatography system was used to quantify histamine in the supernatant

(Shimadzu, Kyoto, Japan). The results were expressed as ng/mL perfusate.

Measurement of CysLTs

CysLTs was quantified by the method of Nagai et al. [23]. A volume of 0.5 mL of perfusate was mixed with 2 mL of ethanol and centrifuged at 8,000 g for 10 minutes at 4°C. The sample supernatant was evaporated, the pH of the residue was adjusted to pH 5.1 using 0.1 N hydrochloric acid, applied to a C-18 column, then washed with 20 mL of distilled water. CysLTs were eluted with 10 ml of ethanol. The effluent was evaporated and CysLTs in the residual solution was measured by an enzyme-linked immunosorbent assay (ELISA) kit (Leukotriene $C_4/D_4/E_4$ EIA System, RPN224; Amersham, Biosciences, NJ, USA). The % cross-reactivity (50 % B/B_0 displacement) for the leukotriene C_4, leukotriene D_4, leukotriene E_4 and leukotriene B_4 antiserum are 100, 100, 70 and 0.3, respectively. The results were expressed as pg/mL perfusate.

Measurement of PGD_2

Perfusate was mixed with 2 mL of ethanol and centrifuged at 8,000 g for 10 minutes at 4°C and the supernatant was evaporated. Samples were adjusted to pH 3 with 0.1 N hydrochloric acid, applied to a C-2 column, washed with 5 mL distilled water, 5 mL 10 % ethanol and 5 mL hexane. PGD_2 was eluted with 5 ml of methyl formate. The effluent was then evaporated, and the amount of PGD_2 in the residual solution was measured by radioimmunoassay (ria) kit (PGD_2 [^{3}H] assay system, TRK 890; Amersham). The % cross-reactivity (50 % B/B_0 displacement) for the PGD_2, PGJ_2 and thromboxane B_2 antisera are 100, 7 and 0.3, respectively. The results were expressed pg/mL perfusate.

Nasal mucosa specimens

Procedures for obtaining nasal mucosa have been described [24]. In brief, 5 hours after the nasal challenge with either histamine or saline, all of the animals were anesthetized and bled. The animals were decapitated, the heads were fixed in 15 % formalin, and the nasal tissue was sectioned and stained with Giemsa to evaluate eosinophil infiltration and with Toluidine blue to evaluate basophilic cell infiltration, respectively. The number of eosinophils or basophilic cells was counted in the whole side of the intranasal epithelium that had the least mechanical injury. The result was expressed as the number of cells per whole side of an intranasal epithelial preparation.

Administration of mediator receptor antagonists

Pyrilamine (10 mg/5 mL/kg) was resuspended in water and administered orally 30 minutes before nasal histamine challenge of sensitized guinea pigs. Atropine (2 μg/100 μL/each nostril) was dissolved in saline and administered 3 hours after nasal histamine challenge of sensitized guinea pigs. Either L-NAME or D-NAME (10 mg/0.5 mL/kg) was administered intravenously 10 minutes before histamine challenge. CGRP (8-37) (0.2 μg/100 μL/kg), a CGRP-1 receptor antagonist, was administered intravenously 2 minutes before histamine challenge. The late phase nasal obstruction appeared 4-6 hours after histamine challenge, therefore, we evaluated the effect of each antagonist when the nasal cavity volume showed the minimum value in the late phase for each comparison.

Statistical analysis

Data are shown as the mean ± standard error (S.E.). Statistical evaluation was performed using Student's t-test corrected by the Bonferroni method for multiple comparisons or the Dunnett test for ANOVA (both Stat View, ver.5.0). A p-value of less than 0.05 was significant.

RESULTS

Effect of histamine challenge on nasal obstruction in sensitized and unsensitized guinea pigs

Both sensitized and unsensitized guinea pigs were given intranasal histamine (4 μg/40 μL/animal). Thirty minutes after nasal challenge of unsensitized guinea pigs (n=11), histamine caused a nasal obstruction resulting in a reduction in nasal cavity volume by 23.4 ± 5.9% of "pre" nasal volume (hereafter designated as -23.4 ± 5.9%). The nasal obstruction improved gradually. In sensitized guinea pigs (n=11), the nasal volume at 30 minutes after histamine challenge was -15.3 ± 5.9% and at 5 hours was -28.1 ± 6.4%. Differences in nasal obstruction between sensitized and unsensitized guinea pigs were significant at 5 hours after histamine challenge, but not after 30 minutes (Figure 1). The repeated experiments revealed that the range of time for the appearance of the late phase nasal obstruction was between 4-6 hours after nasal histamine challenge (data not shown). The peak of the late phase obstruction also fluctuated slightly depending the lots of the experimental animal used. Therefore, in the subsequent experiments, the effects of histamine and drugs were evaluated at the times of the peak of the obstruction, which were 30 min in the early phase and 5 or 6 hours in the late phase, respectively.

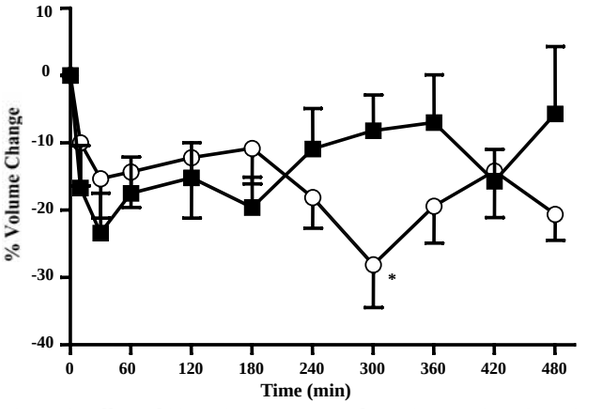

Figure 1. Effect of histamine on volume from the nostril to 2 cm into the nasal cavity in sensitized and unsensitized guinea pigs. The mean baseline values were 0.105 ± 0.006 mL and 0.105 ± 0.005 mL in sensitized and unsensitized animals, respectively. Each point and vertical bar represents the mean ± S.E. of 11 animals. *Significant differences from unsensitized animals at $p < 0.05$. , ○: Sensitized guinea pigs; ■: unsensitized guinea pigs.

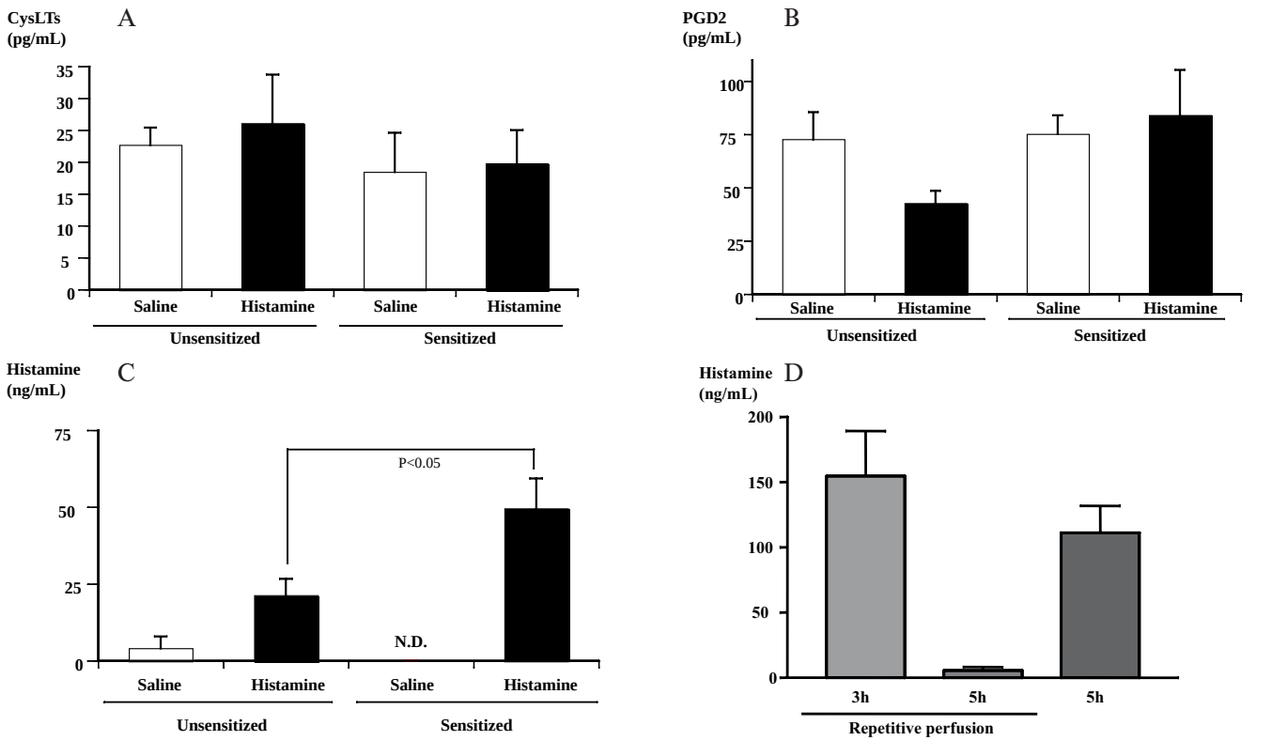

Figure 2. Amount of CysLTs, PGD_2 and histamine in nasal fluids in the late phase induced by histamine or saline nasal challenge of either sensitized or unsensitized guinea pigs. A) CysLTs in nasal fluid. B) PGD_2 in nasal fluid. C) Histamine in nasal fluid. D) Histamine in nasal fluid with and without repeated perfusion. White columns are nasal saline challenge groups, and black columns are nasal histamine challenge groups. Each column and vertical bar represents the mean ± S.E. of 9-10 animals. In A, B and C, guinea pigs nasal perfusion was performed once at 6 hours after either histamine or saline intranasal challenge. N.D.: not detected (under the detection limit).
Repeated nasal perfusions were performed twice at both 3 and 5 hours after histamine challenge in sensitized guinea pigs (D).

Analyses of chemical mediators in intranasal perfusates

The CysLTs, PGD_2, and histamine were quantified in nasal fluids at the late phase following intranasal challenge with either histamine or saline. Neither CysLTs (Figure 2A) nor PGD2 (Figure 2B) levels were significantly different between sensitized and unsensitized guinea pigs. Histamine levels after challenge in sensitized guinea pigs (49.5 ± 9.9 ng/mL) were significantly different from levels in either unsensitized animals (21.2 ± 5.5 ng/mL) or levels induced by saline challenge in sensitized animals (not detected) (Figure 2C). To clarify the origin of the increased histamine in the nasal fluid, the intranasal perfusates were collected repeatedly at 3 and 5 hours after histamine challenge. The nasal fluid collected at 5 hours contained only trace amounts of histamine (2.7 ± 1.4 ng/mL) (Figure 2D).

Histology of nasal mucosa

The eosinophils and basophilic cells that had infiltrated the nasal septum and the nasal turbinate in the late phase were counted (Table 1). The eosinophil and basophilic cell counts in sensitized guinea pigs after either saline or histamine challenge were significantly increased as compared to unsensitized guinea pigs challenged in the same way. However in sensitized guinea pigs, neither the mean eosinophil nor basophilic cell infiltration into the nasal mucosa induced by histamine challenge were significantly different from that induced by saline challenge.

Effects of mediator receptor antagonists

The effects of mediator receptor antagonists on nasal obstruction induced by histamine nasal challenge were tested in sensitized guinea pigs (Table 2). Pyrilamine (10 mg/kg; orally) caused a significant improvement in the nasal obstruction at 30 minutes after the histamine challenge as compared to the water pretreated group. However, pyrilamine had no significant effect on the late phase nasal obstruction. Atropine also had no significant effect on the late phase nasal obstruction. L-NAME (10 mg/kg; intravenously) had no significant effects on the histamine induced nasal obstruction at either 30 minutes or the late phase compared to D-NAME pretreatment group. Intravenous CGRP (8-37) had no effect on the nasal obstruction at 30 minutes after histamine challenge, but did give a significant inhibition in late phase when compared to the saline pretreated group.

DISCUSSION

It is well known that the symptoms of allergic rhinitis occur as a biphasic response, with both early and late phases [25], our

Table 1. Eosinophil and basophilic cell counts in nasal mucosa in the late phase induced by intranasal histamine or saline challenge of sensitized or unsensitized guinea pigs.

		Eosinophils		Basophilic cells	
	Nasal challenge	Nasal septum	Nasal turbinate	Nasal septum	Nasal turbinate
Unsensitized	Saline	162 ± 69	195 ± 36	8 ± 1	9 ± 3
	Histamine	122 ± 58	174 ± 42	12 ± 2	13 ± 2
Sensitized	Saline	1014 ± 260##	1655 ± 373##	26 ± 5##	66 ± 12##
	Histamine	786 ± 189**	1335 ± 254**	25 ± 2**	55 ± 8**

Each value represents the mean±S.E. of the cell number per side of intranasal epithelial preparations (n = 8~10). Guinea pigs were sacrificed at 5 hours after either histamine or saline intranasal challenge. ## Significant differences from saline challenge in unsensitized group at $p < 0.01$. ** Significant differences from histamine challenge in unsensitized group at $p < 0.01$.

data show that a single histamine challenge can produce late phase nasal obstruction in sensitized guinea pigs in the absence of an antigen. It is well known that nasal challenge with histamine cannot evoke a late-phase reaction, but this fact was established using unsensitized animals. No information about nasal obstruction by histamine in sensitized animals in the late phase has been available. In this paper we show that histamine challenge can induce late-phase nasal obstruction in sensitized guinea pigs. Therefore, we investigated this phenomenon to clarify the factors participating in the late phase nasal obstruction induced by histamine.

Table 2. Effects of drugs on nasal obstruction induced by nasal histamine challenge in sensitized guinea pigs.

Drug	Dose	% Volume Change	
		early phase (30 minutes)	late phase (5 or 6 hours)
Water	5 mL/kg	-15.0 ± 3.6	-16.2 ± 3.6
Pyrilamine	10 mg/kg	-6.0 ± 2.1*	-11.1 ± 3.3
Saline	100 μL	-13.3 ± 7.4	-30.1 ± 8.0
Atropine	20 μg/mL	-11.6 ± 5.7	-15.2 ± 7.4
D-NAME	10 mg/kg	-15.6 ± 4.0	-16.2 ± 2.5
L-NAME	10 mg/kg	-12.3 ± 3.7	-9.4 ± 4.0
Saline	100 μL/kg	-22.3 ± 6.2	-22.3 ± 5.7
CGRP(8-37)	0.2 μg/kg	-12.9 ± 6.5	-3.1 ± 4.4*

Each value represents the mean ± S.E. of 6~12 animals. Water and pyrilamine were administered p.o. 30 minutes before nasal histamine challenge. Saline and atropine were administered intranasally 3 hours after nasal histamine challenge. D-NAME and L-NAME were administered intravenously 10 minutes before nasal histamine challenge. Saline and CGRP (8-37) were administered intravenously 2 minutes before nasal histamine challenge. *Statistical differences from contrast group at $p < 0.05$.

Histamine is important in early phase responses [1,2]. Other factors, such as PGD_2 and CysLTs participate in the nasal obstruction accompanying antigen-induced late phase responses [10,26,27]. However, the amount of these mediators in nasal fluid did not increase in our guinea pig models of histamine-induced late phase responses. Histamine caused the release of CGRP and substance P in the peripheral endings of the trigeminal nerve in the nasal mucosa [15,16]. In turn, the released CGRP and substance P induced the release of histamine from mast cells in the nasal mucosa [28]. Our results showed that the amount of histamine in nasal fluid increased in the late phase, but little histamine was detected in sequential repeated perfusates after 3-5 hours. Therefore, the increase of histamine in the late phase in histamine-challenged sensitized guinea pigs is likely to represent the residue of the histamine challenge rather than de novo production by basophilic cells. The clearance of the challenged histamine may decrease in sensitized guinea pigs due to either disturbances in nasal mucociliary transport or enhancement of damage to the nasal epithelium by an antigen-containing spray [29,30].

It is well known that the antigen challenge can induce infiltration of eosinophils and basophils in the late phase response in allergic rhinitis [31-34]. Histamine has chemotactic activity for eosinophils [3,8]. However, in our experimental setting, the effect of histamine challenge on the infiltration of eosinophils or basophilic cells was not observed. Although Giemsa stains not only eosinophils but also neutrophils in guinea pigs [35], we interpreted the staining signal as mainly eosinophils because this nasal allergy model is well known by the characteristic infiltration of eosinophils and basophilic cells rather than neutrophils, in the late phase [36].

In the present study, the nasal obstruction in the late phase was strongly inhibited by the CGRP-1 receptor antagonist. CGRP is known as the most potent endogenous vasodilator. CGRP may also play a role in the regulation of vasomotor responses [37]. Recent studies have suggested that CGRP has protective effects against tissue damage and inflammatory responses [38-42], and Gawin et al. reported that the peptide may enhance plasma extravasation, albumin exudation, and glandular secretion in guinea pigs, and that these mechanisms possibly contribute to nasal responses to injury in this species [42]. Histamine stimulates the trigeminal nerve, including CGRP, via histamine H_1 receptors [43], resulting in the antidromic release of CGRP from terminals of the nerve at 1-3 hours after the stimulation in naive guinea pigs [18]. Furthermore, repeated toluene diisocyanate nasal challenges result in enhancement of the biosynthesis of CGRP in the trigeminal nerve [44]. Therefore, the repeated nasal antigen challenge may enhance the biosynthesis of CGRP in the trigeminal nerve. Nasal hista-

mine challenge to the sensitized guinea pigs could cause release of CGRP from terminals of the trigeminal nerve for a longer time and/or in larger quantities than unsensitized animals. In this scenario, CGRP-induced vasodilatation in nasal mucosa would play an important role in late phase nasal obstruction. Histamine plays an important and direct role in the early phase nasal obstruction, but in the late phase, it would be acting as a trigger for CGRP release rather than a direct effector. However, further studies are necessary to clarify the mechanisms that the release of CGRP induced by histamine in sensitized guinea pigs.

Stimulation of trigeminal nerves is transmitted to the parasympathetic nervous system [45]. Nitric oxide, a transmitter in the parasympathetic nervous system, causes the nasal obstruction [46]. However, L-NAME (an inhibitor of nitric oxide synthetase) and atropine (an anticholinergic drug) did not inhibit the nasal obstruction in the late phase. Therefore, in this model, the parasympathetic nervous system may not play an important role in the late phase nasal obstruction.

Nasal obstruction is a distressing symptom for patients with allergic rhinitis. Our findings point to the importance of CGRP induced by histamine in the late phase nasal obstruction in the animals with hypersensitivity. Histamine-induced CGRP may be clinically important because nasal antigen challenge to patients with allergic rhinitis evokes a 1.5- to 4-fold increase in CGRP for 15 minutes-24 hours, when compared to normal controls [47]. Regulation of either the release or function of CGRP may be a useful therapeutic approach for the suppression of nasal obstruction in allergic patients with hypersensitivity.

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