ANNIKA LUUKKAINEN

Inflammatory Airway Diseases

The impact of Indoleamine 2,3-dioxygenase and outcomes of maxillary sinus surgery

ACADEMIC DISSERTATION
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UNIVERSITY OF TAMPERE

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To my grandfather Tapani,

ABSTRACT

Chronic rhinosinusitis (CRS), allergic rhinitis and asthma are common conditions, with prevalences between 1-20 % in the general population. All three have significant socioeconomical impact on society. Very often, patients with either CRS or allergic rhinitis have asthma and vice-versa. These diseases are linked by partly similar pathomechanisms, which are still not completely known. Disease management aims to alleviate symptoms and is unspecific. Sinus surgery is considered when conservative therapy fails to achieve sufficient disease control, in the treatment of CRS. The golden standard of endoscopic sinus surgery is uncinectomy and middle meatal antrostomy. As of the moment, very few randomized controlled studies are available comparing traditional functional endoscopic sinus surgery (FESS) to minimally invasive endoscopic sinus surgery.

Indoleamine 2,3-dioxygenase (IDO) is an immunomodulatory intracellular enzyme, which may have an impact on these diseases. IDO is present in many cell types. IDO expression has not been studied before in CRS, nasal polyps or allergic rhinitis. IDO activity has not been studied before in asthmatics. Interesting and partly contradictory results have been found on IDO expression in asthma. This thesis aims to investigate if IDO can have a role in the pathogenesis and evolution of the above diseases and to compare the subjective and objective outcomes of FESS and minimally invasive endoscopic sinus surgery.

High IDO expression in the sinonasal mucosa was found in polyp tissue from patients with CRS compared to healthy nasal mucosa. We did not find differences in epithelial IDO expression in the maxillary sinus mucosa between CRS patients and healthy controls. In the nasal mucosa of patients with birch pollen allergic rhinitis and in healthy controls, there were no differences in IDO expression between atopics and healthy controls, during either natural allergen exposure season (spring) and winter. Serum kynurenine to tryptophan ratio (IDO activity) remained unchanged in asthma. Low serum IDO activity was found in atopy and nasal polyps.

In a prospective single-blinded controlled study, simple uncinectomy was randomized on one side of each patient and uncinectomy with additional middle meatal antrostomy was randomized to the contralateral side. Endoscopically evaluated, chronic rhinosinusitis inflammatory changes lessened more quickly on the side with additional middle meatal antrostomy. There were no statistically significant differences between procedures after one month postoperatively. In patients with risk factors, such as asthma or job exposure, choice of operative procedure may be especially important.

In the future more studies are needed on chronic airway inflammation disease subtypes and markers. These might have significance in both pathophysiology and in response to clinical therapy.

LYHENNELMÄ

Krooninen rinosinuiitti, allerginen nuha sekä astma ovat yleisiä terveydellisiä ongelmia, joiden esiintyvyys on 1-20 %. Kaikki kolme tautia aiheuttavat merkittäviä kustannuksia. Varsin usein potilailla, joilla on allerginen nuha tai krooninen rinosinuiitti, on myös astma ja toisin päin. Näitä tauteja yhdistää osittain samanlainen patofysiologia, jota ei vieläkään kunnolla tiedetä. Hoito tähtää useimmiten oireiden helpottamiseen ja on epäspesifistä. Sivuonteloleikkausta harkitaan silloin, kun kroonisen rinosinuiitin konservatiivinen hoito ei riitä. Tavanomaiseen poskionteloleikkaukseen kuuluvat unkinektomian ja keskikäytäväantrostomia-aukon teko. Toistaiseksi on hyvin vähän satunnaistettuja ja kontrolloituja tutkimuksia jotka vertailevat perinteistä ja säästävää endoskooppista poskionteloleikkausta.

Indoleamiini 2,3 dioksygenaasi (IDO) on monessa solutyypissä esiintyvä entsyymi, joka muokkaa immuunijärjestelmää. IDO:n esiintyvyyttä ei ole aiemmin tutkittu kroonisessa rinosinuiitissa, nenäpolyypeissä tai allergisessa nuhassa. IDO:n aktivisuutta ei ole myöskään aiemmin tutkittu astmassa. Väitöskirjatutkimuksen avulla haluttiin selvittää IDO:n yhteyttä näihin kolmeen sairauteen. Lisäksi haluttiin tutkia kroonisen rinosinuiitin operatiivista hoitoa.

Immunohistokemiallisilla värjäyksillä osoitimme, että kroonista rinosinuiittia sairastavien potilaiden polyyppien epiteelissä esiintyi enemmän IDO:a kuin terveessä nenän limakalvossa. Sen sijaan allergista nuhaa sairastavien ja terveiden verrokkien nenän koepaloissa ei havaittu eroja IDO:n ekspresiossa. Kynureniinin ja tryprofaanin suhde (IDO:n aktiivisuus) seerumissa ei eronnut astmaa sairastavien ja verrokkien välillä. Matala seerumin IDO:n aktiivisuus havaittiin atopiassa ja nenäpolyyppeissä.

Prospektiivisessa yksinkertaisesti sokkoutetussa kontrolloidussa tutkimuksessa satunnaistettiin kroonista rinosinuiittia sairastaville potilaille toiselle puolelle säästävä leikkaus, jossa poskintelon aukon, ostiumin edessä olevaa luuta osittain poistettiin (unkinektomia). Toiselle puolelle tehtiin unkinektomia ja lisäksi poskiontelon aukon laajentaminen (keskikäytävä-antrostomia). Noin kuukausi leikkauksen jälkeen endoskooppisesti havaittu leikkausalueen limakalvoturvotus oli vähäisempää antrostomiapuolella. Kun leikkauksesta oli kulunut yli kuukausi, toimenpiteiden välillä ei ollut tilastollisesti merkitseviä eroja oireissa tai endoskooppisissa löydöksissä.

Jatkossa tarvitaan lisää tutkimuksia kroonisten hengitystieinflammaatioiden alatyypeistä ja markkereista. Näillä on todennäköisesti merkitystä sekä patofysiologiassa että hoitovasteessa.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, referred to in the text by their roman numerals I-V.

I Indoleamine 2,3-dioxygenase expression in chronic rhinosinusitis subgroups and antrochoanal polyps. Honkanen T. Luukkainen A. Lehtonen M. Paavonen T. Karjalainen J. Hurme M. Myller J. Huhtala H. Rautiainen M. Toppila-Salmi S. *Rhinology* 2011 Aug;49(3):356-63.

II Indoleamine 2,3-dioxygenase expression in patients with allergic rhinitis: a case-control study. Luukkainen A. Karjalainen J. Honkanen T. Lehtonen M. Paavonen T. Toppila-Salmi S *Clinical and Translational Allergy* 2011 Dec 12;1(1):17.

III Relationships of Indoleamine 2,3-dioxygenase activity and co-factors with asthma and polyps. Luukkainen A, Karjalainen J, Hurme M, Paavonen T, Huhtala H, Toppila-Salmi S. *AJRA 2014* Jan-Feb;28(1):e5-10. doi: 10.2500/ajra.2014.28.4013.

IV Endoscopic Sinus Surgery with Antrostomy Has Better Early Endoscopic Recovery in Comparison to the Ostium-Preserving Technique. Luukkainen A, Myller J, Torkkeli T, Rautiainen M, Toppila-Salmi S. *ISRN Otolaryngology* 2012 Jun 18;2012:189383.

V Satisfaction with maxillary sinus surgery might be influenced by risk factors. Myller J, Luukkainen A, Huhtala H, Torkkeli T, Rautiainen M, Toppila-Salmi S. *Allergy and Rhinology (Providence).* 2013 Spring;4(1):e6-e12. *

***** The University of Tampere has granted approval that this study be also part of Jyri Myller's thesis.
The publishers of the original articles have kindly granted permission to reprint the papers.

ABBREVIATIONS

ACP antrochoanal polyp
AR allergic rhinitis
ARIA Allergic rhinitis and it's impact on asthma
APC antigen presenting cells
CD+ Cluster of differentiation expressing
CF cystic fibrosis
COPD chronic obstructive pulmonary disease
CRS chronic rhinosinusitis
CRSsNP chronic rhinosinusitis without nasal polyps
CRSwNP chronic rhinosinusitis with nasal polyps
CT computed tomography
DC dendritic cell
EPOS 2012 European position paper on rhinosinusitis and nasal polyps 2012
ESS endoscopic sinus surgery
EMT epithelial mesenchymal transition
FESS functional endoscopic sinus surgery
FEV1 forced expiratory volume during the first second of expiration
GCN2-kinase general control nonderepressible 2 kinase
GERD gastro-eosophageal reflux disease
GINA 2014 the Global initiative for asthma 2014
GWAS genome-wide association study
ICS Inhaled corticosteroid
IDO indoleamine 2,3- dioxygenase
IFN- interferon-
Ig- immunoglobulin
IL- interleukin
Kyn kynurenine
LM Lund-Mackay
mAb monoclonal antibody
MCC mucociliary clearance
NAR non-allergic rhinitis
NERD non-steroidal anti-inflammatory drug exacerbated respiratory diseases
NP nasal polyp
NSAID non-steroidal anti-inflammatory drug
OMC ostiomeatal complex
PAS Periodic acid-Schiff
PEF peak expiratory flow
PCD primary ciliary dyskinesia
pDC plasmacytoid dendritic cell
TDO tryptophan 2,3-dioxygenase
TGF Transforming growth factor
Th- T-helper cell
TLR Toll-like receptor
Treg regulatory T cell
Trp tryptophan

1. INTRODUCTION

Allergic rhinitis (AR), nonallergic rhinitis (NAR), chronic rhinosinusitis (CRS), asthma and chronic obstructive pulmonary disease (COPD) are chronic inflammatory airway diseases. They form a heterogeneous, multifactorial group affecting upper and/or lower airways. Of these, the thesis will focus on pathophysiological mechanisms of AR, CRS and asthma in adult patients. AR, CRS and asthma have an increasing prevalence worldwide. They partly associate to each other and to other allergic diseases. They impact significantly patients' lives socially, emotionally, affect sleep, school and work productivity. Each disease economically affects society. CRS has been found to have a greater impact on social functioning than chronic heart failure, back pain or angina (Fokkens et al. 2012). In the US, the annual costs for treating a CRS patient are of 2609 dollars; in Europe the direct annual costs of a severe CRS patient treated in a university hospital are 1861 dollars (Fokkens et al. 2012). AR, CRS and asthma that are not in control or that are progressive in their nature, greatly increase suffering and costs. In the US in 2005, the total estimated annual cost for treating AR is 11.2 billion dollars (Meltzer, Bukstein 2011). Although mortality is now infrequent from acute asthma attacks, asthma patients still account for approximately 10 million office visits, 400 000 hospitalizations and 200 000 emergency room visits in the US (Locksley 2010). The annual economic cost of asthma is estimated at 20 billion US dollars (GINA 2014).

2. REVIEW OF THE LITERATURE

2.1 Anatomy and physiology of the airways

The airways can be divided depending on structure and function. The upper airways comprise the nasal cavity, paranasal sinuses, pharynx until the larynx, at which point the upper airways become the lower airways. The lower airways comprise the trachea, bronchi, bronchioles and alveoli. Physiologically, the conducting zone comprises the nasal cavities, until the bronchioles and serves to moisten, warm, clean inhaled air and senses smell (Tu, Inthavong, Ahmadi 2013,Ganesan, Comstock & Sajjan 2013). The lower respiratory zone comprises respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli, and its function is gas exchange and oxygenation of blood (Tyler 1983, Paulev & Zubieta online).

The bony anatomy of the nose and paranasal sinuses is variable between people. The nose is made of a bony upper part and a completely cartilaginous lower part. Two nostrils are separated by the septum. In each nasal cavity, are three turbinates, attached to the lateral wall of the nose and underneath which are air passages, which force the air to circulate in a steady flow and which maximize cilia and epithelium contact with air. The paranasal sinuses are a collection of four paired air-filled cavities surrounding the nasal cavity. Sinuses are named according to the facial bone in which they are located. The maxillary sinus connects to the nose by an ostium located underneath the middle turbinate (i.e. middle meatus). The frontonasal duct drains the frontal sinus with an ostium in the middle meatus. The ethmoidal sinuses are a collection of variable amount and size air chambers. The posterior group has draining ostia usually into the superior meatus, the middle and anterior group open into the middle meatus. The sphenoidal sinus connects to the nasal cavity via ostia on the posterior wall of the sphenoethmoidal recess, above the choana. (Tu, Inthavong, Ahmadi 2013, O'Rahilly et al. 2004).

The lower airways begin from the glottis, under which is the trachea, which bifurcates into two bronchi at its caudal end. The paired bronchi undergo further branching within the lungs and give rise ultimately to distributing bronchioles. The bronchioles branch further and give birth at their terminal end to the respiratory zone. (O'Rahilly et al. 2004)

2.2. Allergic rhinitis

Allergy (type I hypersensitivity) is the clinical manifestation of an immune response against foreign protein molecules, known as allergens, which are potent inducers of IgE synthesis (Shakib, Ghaemmaghami & Sewell 2008).

Allergic rhinitis is an inflammatory disease of the nasal mucosa caused by an IgE-mediated response in allergen-sensitized subjects (Zhang, Zhang 2014). Another major group of chronic rhinitis is non-allergic rhinitis (NAR). NAR is a heterogeneous syndrome characterized by rhinitis symptoms caused by a non-IgE mediated mechanism regrouping multiple subgroups (Van Gerven et al. 2014, Settipane, Kaliner 2013). According to the Allergic Rhinitis and it's Impact on Asthma global group (ARIA), allergic rhinitis by its' symptoms can be divided into perennial and seasonal forms, the perennial form being more or less a form with continuous symptoms. The current recommendation is to use the terms "persistent" and "intermittent" allergic rhinitis. Intermittent refers to under four symptomatic days per week and/or under four symptomatic weeks per year. Intermittent forms of allergic rhinitis are most often caused by trees, grasses and weeds. Perennial forms, on the other hand, are usually caused by animal dander, dust mite and moulds (Bousquet et al. 2008).

2.2.1 Epidemiology of AR

The prevalence of allergic rhinitis has continually increased in developed countries. Currently, it affects up to 40% of the worldwide population (Bousquet et al. 2008), 23-30% of the European population is affected (Settipane, Peters & Chiu 2013), 12-30% of the US population is affected (Nathan et al. 2008). The prevalence of AR in China depends on the region where an individual lives, ranging from 8.7% in Beijing to 24.1% in Urumqi (Zhang, Zhang 2014). Both Western and non-western countries observe the same increasing trend in incidence.

2.2.2. Genetics of AR

Segregation and twin studies have demonstrated the clear hereditability of AR. Genome Wide Association Studies (GWAS) have identified a total of 22 loci associated with AR (Ramasamy et al. 2011, Hinds et al. 2013, Bunyavanich et al. 2014). Identified loci associate to epithelium, immune system and mitochondrial function. A GWAS on allergen specific IgE level found 10 loci associated with allergen specific IgE level, which accounted for a 25% population attributable risk for AR (Bonnelykke et al. 2013).

2.2.3. Pathophysiology of AR

2.2.3.1 Sensitization

Antigen recognition and uptake by antigen presenting cells (APC), such as dendritic cells (DC), is the first step in the process of antigen presentation that can lead to the initiation of adaptive immune responses. Immature DCs are present in peripheral tissues and can efficiently sample the microenvironment

for antigens. Antigens are processed into peptides and presented on the surface of DCs in major histocompatibility complex molecules. DCs bearing antigen, as previously described, migrate to local lymph nodes, where through expression of the major histocompatibility complex II-peptide complex, cytokines and other co-stimulatory molecules can stimulate naïve T cells towards distinct T cell subsets, T-helper 1 cell (Th1), T-helper 2 cell (Th2), T-helper 17 cell (Th17), or induce tolerance through induction of regulatory T cells (Tregs), depending on nature of antigen and other microenvironmental factors (Salazar, Ghaemmaghami 2013). Thus activated Th2 cells produce interleukins 14 and 13 (IL-4 and -13), which act on mature IgM- and IgD- bearing B cells, circulating in peripheral blood and induce class switch to IgE. In sensitized patients, allergen-specific IgE memory can be strongly boosted by contact with small amounts of allergen via the respiratory tract. The existence of memory IgE cells or long-lived IgE plasma cells and a pool of IgG memory B cells, with specificity for allergens that switch to IgE production upon allergen contact, is currently under debate (Eckl-Dorna, Niederberger 2013). It might be possible that inhaled allergens be directly and rapidly presented also via airway epithelial cells to intraepithelial lymphocytes. (Westendorf et al. 2006, Golebski et al. 2013).

2.2.3.2. Allergen transport and intercellular junctions

Allergen entry depends on several factors in host, environment and allergen itself, which are not fully understood. Partly controversial evidence exists whether aberrant epithelial absorption of allergens associates with allergy phenotype (Georas, Rezaee 2014a, Post et al. 2013, Hackett et al. 2013, Clarke et al. 2014,Greiff et al. 2002, Passalacqua et al. 2005).

Pollens have been shown to down-regulate the antiproteases contained in mucus and in the mucosa, to inflict direct damage to the epithelium itself by either lytic activity, liberation of substances acting as pro-inflammatory mediators and to disrupt epithelial tight junctions (Runswick et al. 2007). On the other hand, birch pollen allergen has been shown to be taken in and transported inside epithelium by active caveolar transport, which occurred only in subjects allergic to birch pollen (Joenvaara et al. 2009). In addition, some allergens find their way directly into the bloodstream (Golebski et al. 2013). Certain allergens, by destroying epithelial integrity, can pass through tight junctions directly into the lamina propria, without necessiting a transport mechanism (Golebski et al. 2013). It has also been demonstrated that DCs with cytoplasmic extensions seem to be able to reach within the airway lumen and take in the allergen directly, without the need for the allergen to pass through the epithelium by separate transportation means (Sung et al. 2006, Zimmerli, Hauser 2007, Kojima et al. 2013).

2.2.3.3. Aberrant barrier immunity in AR

The sinonasal barrier has an active role in both innate and adaptive immunity. Alterations in several barrier functions, such as antimicrobial substance release, pattern recognition, cytokine release, immune and neural cell activation, antigen presentation and phagocytosis, have been shown to associate with the allergic response (Golebski et al. 2013). As an example, Toll-like receptors (TLR) 1-10 are widely expressed in nasal epithelium (Fokkens et al. 2012). They not only recognize molecular patterns of pathogens and damaged host cells, but also allergens thus possibly resulting in the aggravation of allergic inflammation (Golebski et al. 2013, Fokkens et al. 2012, Tengroth et al. 2014, Starkhammar et al. 2014). In addition, epithelial cytokines, Thymic stromal lymphopoietin and IL-33, have been shown to activate Th2 responses and to associate with the allergic airway response (Scadding 2014).

2.2.3.4. Elicited allergic responses

Allergic rhinitis is subdivided into the acute and chronic phase. The early phase response is the period including the first minutes to about an hour after allergen challenge (Bousquet et al. 2008). In sensitized individuals, the epitope of the transported allergen crosslinks with membrane bound allergen specific IgE, bound to high-affinity Fc-ε receptors on the surface of mediator cells, such as mast cells and basophils. This results in immediate release of allergic mediators, (both pre- and newly formed) including histamine, tryptase, cysteinyl leukotrienes, eosinophilic cationic protein, prostaglandin D2 as well as neuropeptides (substance P, vasoactive intestinal polypeptide) which are responsible for the immediate allergic response, including sneezing, rhinorrhea and nasal itching (Scadding 2014, Min 2010, Prussin, Metcalfe 2006, Kay 2001). During the early phase response, Th2 cytokines remain at pre-allergen challenge levels.

The late phase response is the period starting from 3-4 hours after allergen challenge and lasting at least 24 hours (Bousquet et al. 2008). It is locally characterized by a Th2 inflammatory pattern with influx of mast cells, eosinophils, basophils, IgE expressing B cells and T cells. These cells migrate to site of inflammation through endothelial cells, stimulated by freed allergic mediators to express adhesion molecules to leukocytes (Bousquet et al. 2008). Nasal obstruction is the main symptom of the late allergic response (Rondon et al. 2012, Greiner et al. 2011). During this time, IL-4,-5 and -13 can be detected in nasal fluid, reaching a high at 6-9 hours after challenge and returning to baseline levels 24 hours after challenge (Scadding 2014).

Environmental exposure may have varying effects in allergic responses (Eggleston 2009). Both human and animal nasal models have demonstrated that for instance diesel exhaust particles can act as a stimulant to amplify allergic

immune responses (Evans et al. 2014a, Nikasinovic, Momas & Just 2004, Bleck et al. 2006, Bleck et al. 2008).

2.2.4 Clinical presentation of AR

Typical symptoms, in the acute phase, shortly after contact with the offensive allergen include rhinorrhea, nasal pruritus, nasal obstruction, impaired sense of smell, sneezing and are often accompanied by ocular pruritus, redness and/or lacrimation in 60-70% of patients (Bousquet et al. 2008, Canonica et al. 2007, Schatz 2007).

2.2.5.Diagnostic criteria of AR

Diagnosis of allergic rhinitis can be made following the criteria established by ARIA. These criteria include a patient history with typical symptoms following allergen challenge concordant with positive diagnostic tests. Diagnostic tests include skin prick tests with both positive and negative control solutions and measurement of allergen specific serum IgE. Recent interest has been taken into local allergic rhinitis, with only local IgE formation and no skin-prick test positivity or allergen-specific IgE detection. It has been proposed to be a new disease entity, local AR, or to be a form of developing AR (Rondon et al. 2012). Unspecific findings of allergic rhinoconjunctivitis are bilateral conjunctival swelling and erythema, eyelid swelling, lower eyelid venous stasis, swollen nasal turbinates, and watery nasal fluid (Bousquet et al. 2008).

2.2.5.1. Associated comorbidities and differential diagnosis

AR associates to other allergic diseases: allergic conjunctivitis, atopic asthma, atopic dermatitis and food allergy. Due to the fact that prevalence of AR is elevated, it might mask a condition overlapping AR or, might exist as a distinct entity mimicking rhinitis. These conditions include inflammatory conditions of the sinonasal tract (such as CRS, NAR) and the neighbouring organs (adenoiditis); foreign body, structural abnormalities, tumours, and cerebrospinal fluid leak (Bousquet et al. 2008, Fokkens et al. 2012).

2.2.6.Clinical management

Following ARIA guidelines, the management of allergic rhinoconjunctivitis encompasses patient education and pharmacotherapy, which consists of non-sedating second-generation oral H1-antihistamines, intranasal corticosteroids, chromones and leukotriene antagonists. Allergen-specific immunotherapy may alter the natural course of allergy for several years and may reduce the risk of developing asthma (Moller et al. 2002, Jacobsen et al. 2007). It is considered if

airborne allergy is not managed with medication and it is persistent and/or moderate to severe in symptoms (Bousquet et al. 2008b).

2.3. Chronic rhinosinusitis

As defined by the European position paper on rhinosinusitis and nasal polyps 2012 (EPOS 2012), chronic rhinosinusitis is a multifactorial complex disorder involving inflammation of the nose and paranasal sinuses lasting at least 12 weeks (Fokkens et al. 2012). CRS is most often classified into chronic rhinosinusitis with nasal polyps and without. Controversy still remains about classification of CRS into new endophenotypes, with potentially different pathogenetic mechanisms and/or therapeutic responsiveness. CRS can also be classified according to histologic evidence of chronic hyperplastic eosinophilic sinusitis (increased sinonasal tissue eosinophilia) or chronic inflammatory sinusitis. Under this classification system, nasal polyps may be associated to either chronic hyperplastic eosinophilic sinusitis or chronic inflammatory sinusitis(Fokkens et al. 2012).

2.3.1. Epidemiology of CRS

The first European population-based study on CRS found a prevalence of 10.9% (Tomassen et al. 2011). In Korea, a population-based survey came to a prevalence of only 1% in 1991, which rose to 7%, when results were reported again in 2011(Min et al. 1996, Kim et al. 2011). CrswNP affects 1-4% of the population (Settipane, Peters & Chiu 2013). Both CRSsNP and CRSwNP tend to affect adult populations. Their prevalence increases with age until 60 years. CRSsNP affects women more than men, whereas CRSwNP affects more likely men than women. NPs are extremely uncommon in patients under the age of 20 (Fokkens et al. 2012).

2.3.2. Genetics of CRS

Inheritance of CRS fails to be documented yet, as with AR and asthma. Genetic basis of CRS is strongly suspected; reports of families with unusually high prevalences of CRSsNP and CRSwNP exist (Hsu et al. 2013). Two GWAS are available, based on DNA sample pools. Gene association to basement membrane formation, host defence against bacterial lipopolysaccharide (acyloxyacyl hydrolase) were found (Bosse et al. 2009, Hsu et al. 2013). Genetic variability was found in innate immunity genes and inflammatory mediators (including IL-13 and -33). Moreover, genes involved in tissue remodeling and arachidonic acid metabolism may be involved in CRS pathogenesis (Hsu et al. 2013).

2.3.3. Pathophysiology of CRS

2.3.3.1. Mucociliary clearance in CRS

The nasal epithelium consists mainly of ciliated and non-ciliated columnar cells, goblet cells and basal cells as well as olfactory cells (Watelet et al. 2006). Paranasal sinus epithelium consists of fewer ciliated and goblet cells than nasal epithelium (Watelet et al. 2006). Airway mucus contains more than 200 proteins and is secreted both by goblet cells and submucosal glands. Mucus has antioxidant, antiprotease and antimicrobial activities. In healthy individuals, circadian rhythms regulate normal mucus secretion, principally through the vagus nerve (Rose, Nickola & Voynow 2001, Thornton, Rousseau & McGuckin 2008). Efficient transport of mucus is dependent on the rate of coordinated ciliary beatings, as well as the hydration of mucus (Kilburn 1968, Puchelle, de Bentzmann & Zahm 1995, Tarran et al. 2001). Cilia are complex organelles distributed throughout the human body. Motile cilia are responsible for the transport of extracellular fluid, 200-300 cilia are present apically on each ciliated cell (Serafini, Michaelson 1977).

Impairment of mucociliary clearance (MCC) leads to mucostasis, bacterial colonization and biofilm formation and thus predisposes to chronic infections of the upper respiratory tract (Moller et al. 2006, Zhang et al. 2013). Li et al performed a culture of epithelial stem cells from patients with CRSwNP and without a diagnosis of primary ciliary dyskinesia (PCD). The group found aberrant ciliogenesis, which might indicate that primary motile cilia impairment might associate more frequently with CRSwNP than previously thought (Li et al. 2014)

2.3.3.2 Microbes and biofilms in CRS

Biofilms are surface-associated microbial communities, which provide protection from antimicrobial substances (Donlan, Costerton 2002, Desai, Mitchell & Andes 2014). Biofilms have been extensively studied during the past years as a potential mechanism for the development of CRS and aggravation and persistence of CRS symptoms (Korkmaz et al. 2014). Mucosal biofilms are a marker of more severe mucosal disease and a predictor of poorer outcome after sinus surgery (Hochstim, Masood & Rice 2010, Hamilos 2014). *Staphylococcus aureus* and *Pseudomonas aeruginosa* are commonly detected in diseased sinus tissue, as well as their biofilms, in more severe cases (Hamilos 2014). In addition, *S. aureus* can produce staphylococcal exotoxins that can initiate/maintain a Th2 response and can form enterotoxins with superantigenic properties (Akdis et al. 2013). In humans, superantigens have the ability to induce a polyclonal IgE response against multiple allergens (Corriveau et al. 2009, Akdis et al. 2013). Antibiotic exposure and concomitant asthma have

been found to be associated with reduced microbial diversity and increased *S. aureus* abundance, in CRS patients (Hamilos 2014).

The majority of patients with CRS have also fungal hyphae detectable in mucus extracted from diseased sinuses, in association with bacterial biofilm (Hamilos 2014, Akdis et al. 2013).

2.3.3.3. Immune responses in CRS

Variable immune responses have been detected in CRSsNP and CRSwNP phenotypes, probably due to both genetic and environmental factors (Akdis et al. 2013). Yet their role in the pathogenesis of CRS has not been resolved (Toppila-Salmi et al. 2015). Decreased presence or function of epithelial antimicrobial enzymes, (lysozyme and lactoferrin), host defence molecules (psoriasin/S100A7) lipopolysaccharide-binding antimicrobial peptides, and tight junction proteins, have all been reported to associate with CRS (Akdis et al. 2013). Reduced transepithelial resistance, and TLR responses (particularly TLR2 and TLR9) associate with CRSwNP in comparison to CRSsNP and healthy controls (Akdis et al. 2013, Tengroth et al. 2014). Several cytokines have been shown to associate with CRS, such as IL-1, tumour necrosis factor-alpha (TNF-alpha), IFN-gamma, granulocyte macrophage colony-stimulating factor, eotaxins, IL-6, IL-8, osteopontin, thymic stromal lymphopoietin (Fokkens et al. 2012, Van Zele et al. 2014). Moreover, IL-5 and IL-17 associate with eosinophilia and CRSwNP, (Bachert et al. 2010, Zhang et al. 2008). T-, B- and plasma cells have been detected. These are associated with increases in local production of several immunoglobulin isotypes, especially IgE and IgA. Total IgE levels in nasal polyps are often highly increased, independently of atopy and related to the degree of eosinophilic inflammation (Akdis et al. 2013). Increased specific autoantibodies, IgG deposition and IgA-secreting plasma cells associate with nasal polyp tissue when compared to control and CRSsNP subjects (Tan et al. 2011).

2.3.3.4. Epithelial remodeling in CRS

Nasal epithelial repair and remodeling is a normal process of epithelial fast self-renewal (Hupin et al 2014, Toppila-Salmi et al. 2015). It is a highly organized and well-coordinated process, involving inflammation, proliferation, differentiation, matrix deposition, and remodeling, and is regulated by a wide variety of growth factors and cytokines (Yan, Gordon & Wang 2013). Epithelial cells terminally differentiate (e.g. ciliated cells, goblet cells) from basal cells that possess stem cell characteristics. Epithelial cells may dedifferentiate through squamous metaplasia, in which they lose cell-to-cell polarity and various other features whilst acquiring mesenchymal features such as vimentin filaments (Kalluri, Weinberg 2009). This so called epithelial-to-mesenchymal transition

(EMT), occurs during normal development and wound repair (Hupin et al 2014, Toppila-Salmi et al. 2015).

Distinct remodeling patterns associate with the severity of CRS and with CRS phenotypes. Partly similar remodeling changes are seen in both upper and lower airways (Fokkens et al. 2012, Hupin et al. 2014, Shi et al. 2013, Meng et al. 2013). Aberrant remodeling processes associating with CRS include fibrosis, epithelial alterations, basement membrane thickening, goblet cell hyperplasia, sub-epithelial oedema, inflammatory cell infiltrates, and angiogenesis (Fokkens et al. 2012, Toppila-Salmi et al. 2015). Aberrant remodeling has been detected also in parallel to inflammation and not only as a consequence of inflammation (Fokkens et al. 2012, Meng et al. 2013, Dhong 2012).

2.3.4. Clinical presentation of CRS

As defined by EPOS 2012, CRS typically presents with the following local symptoms: nasal blockage or congestion, nasal discharge or postnasal drip, facial pain or pressure, reduction or loss of smell. In addition to these, often distant symptoms including pharyngeal, laryngeal and tracheal irritation can manifest. Acute exacerbations may be recognized by more severe symptoms and a more rapid progress of symptoms (Fokkens et al. 2012).

2.3.5. Diagnostic criteria of CRS

In primary care settings, CRS is diagnosed by the following symptoms; one of which should either be nasal blockage/obstruction/congestion or nasal discharge and/or facial pain/pressure and/or reduction or loss of smell during at least 12 weeks (Fokkens et al. 2012). In secondary and tertiary care, typical signs should also be seen either endoscopically and/ or on computed tomography-scans (CT-scans). Endoscopically the following changes should be seen: nasal polyps and/or mucopurulent discharge and/or oedema/mucosal obstruction of the middle meatus. In CT-scans, CT-changes and/or mucosal changes within the ostiomeatal complex and/or sinuses should be observable (Fokkens et al. 2012).

2.3.5.1 Endoscopic staging

Nasal endoscopy may be performed with and without decongestion. Semi-quantitative scores for polyps, oedema, discharge, crusting and scarring (post-operatively) can be obtained at baseline and following therapeutic intervention, at regular intervals. Nasal endoscopy is superior to anterior rhinoscopy in terms of illumination, examination of middle and superior meati, the nasopharynx and mucociliary drainage pathways (Fokkens et al. 2012).

2.3.5.2. Lund-Mackay staging (CT-scan evaluation)

A number of CT- staging systems of CRS exist, the Lund-Mackay staging is one of the most used. Staging is based on degree of opacification (0=normal, 1=partial opacification, 2= total opacification) of each sinus: maxillary, anterior ethmoid, posterior ethmoid, sphenoid and frontal sinus, for each side. In addition, the ostiomeatal complex is graded as 0=not occluded, or 2= occluded, coming to a maximum score of 12 per side (Fokkens et al. 2012).

2.3.6. Conditions associating or mimicking CRS

The most prevalent CRS mimicking or associating conditions are headaches, inflammatory diseases, and structural abnormalities (Fokkens et al. 2012). Head and facial pain disorders mimicking with CRS include tension type and cluster headache, neuropathic pain or temporomandibular joint dysfunction (Fokkens et al.2012). Inflammatory conditions associating with CRS include airway allergy, inflammation of the oral cavity and GERD (Tan et al. 2013). Structural abnormalities associating with CRS include nasal septal deviation, nasal valve dysfunction, concha bullosa, and adenoid hyperplasia.

It is important to avoid delayed diagnosis of benign and malignant tumours behind treatment-resistant or unilateral symptoms. Tumours in the sinonasal tract include squamous cell carcinoma, lymphoma, inverted papilloma, olfactory neuroblastoma, juvenile angiofibroma and hemangioma. Rare inflammatory comorbidities (vasculitis, sarcoidosis, cystic fibrosis, primary ciliary dyskinesia, immunodeficiencies) must be taken into account especially if a patient presents with other general symptoms (Tan et al. 2013, Akdis et al. 2013, Fokkens et al. 2012). Other rare causes of persisting and worsening facial pain include diseases of the orbital region (e.g. systemic inflammatory diseases, neoplasms, congenital malformations), tumours irritating nerves (e.g. trigeminal nerve) and central lesions (syringobulbia, multiple sclerosis) (Fokkens et al. 2012, Akdis et al. 2013).

2.3.6.1. Antrochoanal polyps

Antrochoanal polyps (ACP) originate from the inner wall of the maxillary sinus, and during their growth either pass through the natural ostia or cause pressure-induced destruction of the medial sinus wall and formation of accessory ostia. They have an incidence of 3-6% in all nasal polyps, and have not been extensively studied. ACP have a much higher incidence in paediatric populations. Histologically, ACP are covered by ciliated cylindrical epithelium. The stroma is oedematous and highly vascular, comprising mainly loose connective tissue infiltrated with plasma cells and a few eosinophils. Pathophysiology of ACP is still under debate, lymphatic eodema and blockage of acinous mucus glands have been proposed as pathomechanisms(Mostafa et al. 2014).

2.3.6.2.Primary and secondary ciliary dyskinesia

Primary ciliary dyskinesia (PCD) is a rare hereditary disease affecting both ciliary ultrastructure and ciliary motility, but not mucus secretion (Noone et al. 2004). Patients typically experience prolonged and recurrent bacterial infections, (acute and chronic rhinosinusitis and asthma in most patients) (Boon et al. 2013, Rollin et al. 2009).

Viral or bacterial infections and exposure to gasses or drugs can cause secondary functional and ultrastructual changes in cilia. These changes are usually reversible, in contrast to PCD (Gudis, Zhao & Cohen 2012).

2.3.6.3. Cystic fibrosis

Cystic fibrosis is a rare hereditary metabolic disease, which impairs normal sodium and chloride transport across the epithelium, causing viscous mucus. Wang et al found that the prevalence of CRS in an unselected group of cystic fibrosis (CF) carriers was of 36%, in comparison to the 10.9-12.5% CRS prevalence in the general population (Wang et al. 2005). CF is one of the most common causes of NP in children. Sinuses bear different bacterial subpopulations, in comparison to healthy individuals, which seem to migrate to the lower airways (Boon et al. 2013).

2.3.7. Clinical management of CRS

2.3.7.1Conservative management of CRS

According to EPOS 2012, intranasal corticosteroids and nasal saline irrigation form the primary management of CRS. Local corticosteroids improve both upper (rhinitis, nasal polyps) and lower (asthma) airway inflammatory conditions (Fokkens et al. 2012). They have special importance in CRSwNP patients in terms of polyp growth prevention. Nasal saline irrigation (isotonic or hypertonic) is beneficial in terms of symptoms relief-(Fokkens et al. 2012). Additional treatment depends on the nature of exacerbations and may include short courses of peroral corticosteroids, antibiotics, antihistamines and decongestants. Short courses of systemic corticosteroids are used when CRSwNP is no longer under control, however side effects and short-term effects limit their use. Antihistamines can be used in patients with concomitant allergic rhinitis (Fokkens et al. 2012).

2.3.7.2. Operative management of CRS

The ostiomeatal complex (OMC) is a functional entity surrounding the middle meatus and maxillary sinus ostium, which consists of the ethmoid infundibulum, hiatus semilunaris, bulla ethmoidalis, uncinate process and middle turbinate. Anatomically, it is a common pathway for ventilation and drainage of maxillary, anterior ethmoid and frontal sinuses. FESS was introduced to correct OMC

occlusion and restore normal physiology to paranasal sinuses (Stammberger 1986a, Stammberger 1986b). Endoscopic sinus surgery has been proven to be safe and effective in the management of CRSsNP patients, when conservative treatment fails. It is not yet fully known whether FESS is superior to maximal conservative therapy (Ragab, Lund & Scadding 2004, Fokkens et al. 2012). FESS is more likely to be effective in alleviating nasal obstructing and facial pain, as well as improving generic and disease-specific quality of life outcomes (Fokkens et al. 2012).

Evidence is substantial now in implicating inflammatory processes over simple obstructive phenomenon in CRS patients. Eosinophilic chronic rhinosinusitis has been shown to have worse disease severity and poorer treatment outcomes compared to non-eosinophilic CRS (Ferguson 2004, Tosun et al. 2010). Snidvongs et al argue that anatomical OMC blockage is unlikely to induce eosinophilic inflammation in these patients and that solely operative procedures manipulating the OMC are unlikely to benefit this subgroup of CRS (Snidvongs et al. 2013). In the majority of cases, functional endoscopic sinus surgery is recommended over polypectomy, Caldwell-Luc, inferior meatal antrostomy and antral irrigations. In CRSwNP, polypectomy alone relieves nasal blockage symptoms. However, recurrence rate is high, especially without aggressive postoperative medical therapy (Fokkens et al. 2012).

2.4. Asthma

Asthma is a heterogeneous and variable syndrome. It is characterized by chronic inflammation causing bronchial hyperreactivity, mucus hypersecretion and airway eodema leading to variable airflow obstruction (Kontakioti 2014, Locksley 2010, Erle, Sheppard 2014). Unsupervised hierarchical cluster analyses have identified clinically distinct phenotypes, such as early-onset atopic, obese noneosinophilic, early-onset symptom predominant, and late-onset inflammation predominant (Haldar et al. 2008, Amelink et al. 2013). More recently, huge variation has been demonstrated also within the mentioned phenotypes, suggesting multiple endotypes of asthma (Simpson et al. 2010, Just et al. 2014).

A subpopulation of patients suffering from asthma can be classified as having NSAID exacerbated respiratory disease (NERD). Within two hours of ingestion of aspirin or other non-steroidal anti-inflammatory drugs (NSAID), these patients develop acute dyspnoea, usually accompanied with nasal symptoms, rhinorrhea and/or nasal congestion. Most of these patients present with the "aspirin triad", which consists of chronic rhinosinusitis with nasal polyps, severe bronchial asthma and intolerance to aspirin and other NSAIDs (Kowalski 2013). Interestingly, patients suffering from NERD have a higher recurrence of polyps following polypectomy and a more extended hypertrophy of the nasal

and paranasal sinus mucosa, in comparison to aspirin-tolerant asthmatics (Fokkens et al. 2012, Laidlaw, Boyce 2013).

2.4.1. Epidemiology of asthma

The estimated worldwide asthma prevalence is of about 300 million people, prevalences between 1-18% have been found in different countries (GINA 2014). Approximately 5-10% of individuals with asthma have severe disease, with symptom persistence despite maximal medical therapy and high rates of exacerbations leading to hospitalization (Brightling et al. 2012).

2.4.2. Genetics of asthma

Genetic predisposition to asthma is clear, family and twin-studies have suggested hereditary contributions approaching 60% (Locksley 2010). So far, GWASes have produced over 27 loci associating with asthma, yet their contribution in asthma pathogenesis is unresolved (Locksley 2010, Ortega, Meyers 2014). Loci that significantly associate to asthma seem to depend on ethnic background and the method used for asthma phenotype characterization (Ortega, Meyers 2014). Most replicated candidate genes associate to innate immunity, Th2 inflammatory pathway signalling, lung development, as well as cellular signalling, metabolism and regeneration processes. Locus 17q12 encodes ORM1-like 3 gene and neighbouring gasdermin-like genes. It has become the most replicated loci for asthma susceptibility (Ortega, Meyers 2014).

2.4.3. Pathophysiology of asthma

2.4.3.1. The role of the airway barrier in asthma pathogenesis

Substantial evidence suggests that the persistence of asthma is driven by ongoing host immune responses that generate mediators driving airway remodeling and airway dysfunction. The epithelium is both a site of production of these mediators as well as source of cells that respond to mediators produced by immune cells and other cells within the airway (Erle, Sheppard 2014). Reduced epithelial tight junction structure and components are associated with asthma (Georas, Rezaee 2014b, Xiao et al. 2011). Patients suffering from asthma might have signs of remodeling and EMT, such as airway wall thickening, epithelial hypertrophy, mucous metaplasia, subepithelial fibrosis, myofibroblast and smooth muscle cell hyperplasia and hypertrophy, increased basement membrane thickness and increased number of transforming growth factor β-1 positive epithelial cells (Honkova et al. 2014, Elias et al. 1999, Tyner et al. 2006, Erle, Sheppard 2014). Airway remodeling is seen both in the

presence and absence of injury and infection (Elias et al. 2003, Erle, Sheppard 2014, Ordonez et al. 2000).

Hypersecretion of mucus from metaplastic and hyperplastic goblet cells contributes to obstruction of airways (Rogers 2003). Aberrant production, cross-linking, hydration, secretion, storage and clearance of mucins have all been found to associate with asthma, even in its mild to moderate forms (Erle, Sheppard 2014, Ordonez et al. 2001). Impaired ciliary motility, dead cells and loss of epithelial structure integrity are associated with asthma (Thomas et al. 2010, Erle, Sheppard 2014). In addition, subjects with asthma have a distinct lower airway bacteriome (such as enrichment of proteobacteria) compared to controls (Rubin et al. 2014).

2.4.3.2. Inflammatory mediators and immune responses in asthma

Many phenotypes of asthma can be considered as being Th2-driven responses, for example early-onset (atopic) asthma, later-onset (eosinophilic) asthma and exercise-induced asthma. Inhaled allergens in sensitized individuals trigger activation of mast cells and subsequent release of leukotrienes and prostaglandins, in the early asthmatic response. The late response is characterized by the release of IL-4, -5, -13 and eotaxin by activated Th2 cells with subsequent IgE isotype switching, eosinophil activation and smooth muscle cell proliferation. Airway epithelial cells also secrete IL-25 and IL-33 which activate DCs and cause innate lymphoid cells to release IL-5 and IL-13 (Cates et al. 2004, Hammad et al. 2009, Locksley 2010, Nagarkar et al. 2012, Erle Sheppard 2014.). Characteristics of Th2 low asthma include Th17 family cytokines (Newcomb and Peebles 2013, Erle Sheppard 2014).

Leukotrienes are produced by many inflammatory cells (eosinophils, macrophages, basophils and mast cells) and are considered as important inflammatory lipid mediators in the pathogenesis of asthma. They are able to recruit and activate eosinophils, increase microvascular permeability and cause secretion of mucus, smooth muscle constriction and proliferation (Mechiche et al. 2003). Deficiency in prostaglandin E2 synthesis might associate with NERD (Liu et al. 2013). Inflammatory mediators for predicting asthma control are currently under tremendous interest. Serum soluble CD30, periostin and calprotectin seem to associate with certain asthma subsets and response to therapy (Delezuch et al. 2012, Jia et al. 2012, Aoki et al. 2009, Parulekar, Atik & Hanania 2014).

2.4.3.3. The role of smooth muscle cells

Excessive airway narrowing takes place during an acute asthma attack. It is largely due to contraction of the bands of smooth muscle in the walls of large- and medium sized conducting airways in the lung. Contraction of smooth

muscle can be physiologically induced by release of acetylcholine from efferent parasympathetic nerves or by release of histamine and cysteinyl leukotrienes, resulting in airway narrowing, with collapse in medium-sized airways (Erle, Sheppard 2014). In healthy individuals, physiological release of any of these three mediators causes only mild and usually asymptomatic airway narrowing. Asthmatics however, have a marked sensitivity to these contractile agonists (Boushey et al. 1980). Aberrant airway smooth muscle remodeling associates with asthma, and may be distinct in eosinophilic and neutrophilic subtypes of asthma (Elliot et al. 2014). Abnormal contraction and relaxation of smooth muscles in asthmatic airways have also been observed, in comparison to controls (Sferrazza Papa, Pellegrino & Pellegrino 2014, Erle, Sheppard 2014).

2.4.3.4. Neurons and asthma pathogenesis

A significant amount of data points out that the nervous system may modulate asthma-symptoms (Barnes 1986, Undem, Carr 2002, Chiu et al. 2013, Bautista et al. 2006). It has been demonstrated in murine models that sensory neurons and neuropeptides mediate allergic hyperconstriction as well as key motility and phagocytosis features of DCs (Trankner et al. 2014, Voedisch et al. 2012).

2.4.3.5. Environmental irritants and microbes in asthma

Several studies have shown that asthmatics have heightened immune-inflammatory responses to air pollutants like PM 2.5-10 and ozone (Evans et al. 2014b). Exposure to diesel exhaust particles has been shown to trigger Th2 immune responses. Increased risk of wheezing or asthma has been detected in children exposed to cigarette smoke or formaldehyde (Auerbach, Hernandez 2012, Rager et al. 2013). Cigarette smoke is associated with aberrant airway epithelium functions and increased allergen penetration (Gangl et al. 2009, Jones et al. 1980, Moerloose, Pauwels & Joos 2005).

2.4.4. Clinical presentation of asthma

According to the Global initiative for asthma 2014 (GINA 2014), symptoms suggestive of asthma are intermittent and varying degree of severity attacks of breathlessness, wheezing, cough and chest tightness. However, these symptoms are unspecific, thus other conditions besides asthma must also be taken into account. These symptoms are often worse at night or in the early morning. Symptoms are triggered by viral infections, exercise, allergen exposure, changes in weather, laughter or irritants, such as car exhaust fumes, smoke or strong smells (GINA 2014).

2.4.5. Diagnosis of asthma

Adult asthma diagnosis is based on documented reversible obstruction in lung function tests in Finland (Astma-kaypa hoito 2012). In spirometry, airflow limitation is present when the ratio of forced expiratory volume during the first second of expiration (FEV1) to forced vital capacity is reduced to about 0.75 to 0.80 (GINA 2014, Astma-kaypa hoito 2012). An increase of FEV1 by at least 12% and 200ml from pre-bronchodilator values constitutes an asthma diagnosis. Asthma diagnosis can also be made after a two-week measurement of Peak expiratory flow (PEF). Significant daily diurnal variability or improvement in obstruction with tested medication, comprise an asthma diagnosis (GINA 2014, Astma-kaypa hoito 2012). Documentation of airflow limitation in a certain range in response to exposure to metacholine, histamine, mannitol, hypertonic saline solution, hyperventilation and exercise, are also used for diagnosing asthma (GINA 2014). Wheezing during normal or forced expiration may support an asthma diagnosis, yet it is unspecific and insensitive for asthma (GINA 2014).

2.4.6. Associated comorbidities and differential diagnosis of asthma

CRS, atopy, and occupational and other environmental factors associate with asthma and its exacerbations (GINA 2014). In addition, the following conditions may be found behind uncontrolled or atypical asthma: hyperventilation syndrome, panic attacks, upper airway obstruction, inhalation of foreign objects, vocal cord dysfunction, pulmonary embolism, other forms of obstructive lung disease (COPD, non-obstructive forms of lung disease and non respiratory causes of symptoms (e.g. heart conditions, GERD). Rare conditions include tumours (trachea and lungs), anatomic anomalies (aortic arch malformation, tracheomalacia), sarcoidosis, CF, PCD, immunodefiencies, and alpha-1-antitrypsin deficiency (GINA 2014).

2.4.7. Clinical management

Due to the nature of asthma, pharmacotherapy is based on daily controller medication with in part anti- inflammatory properties and as-needed reliever medication, which acts promptly to relieve bronchoconstriction and relieves symptoms. In asthmatics failing to achieve control with controller therapy, add-on therapy is available (GINA 2014).

2.5. United airways

Inflammation of the upper airways affects the lower airways and visa-versa. 20-50% of patients with allergic rhinitis and 20-60% of CRS patients have asthma, whereas over 80% of allergic asthmatics have concomitant rhinitis (Bousquet et

al. 2008, Fokkens et al. 2012, GINA 2014). All three conditions are characterized by excessive mucosal inflammation with immune dysregulation, with overlapping pathomechanisms. The airway mucosa, from nose to alveolar units is lined by the same respiratory epithelium, with changes in specific cell proportions (Kariyawasam, Rotiroti 2013, Lin et al. 2011). Concomitant epithelial shedding in the airways may compromise the barrier function and increased susceptibility to bacterial colonization, biofilm formation and continued inflammation (Akdis et al. 2013). Compared to controls, allergic subjects proved to have reduced divergence in epithelial gene-expression fingerprints between the upper and lower airways (Wagener et al. 2013).

2.6. Indoleamine 2,3 dioxygenase

2.6.1. Tryptophan degradation and Indoleamine 2,3-dioxygenase

Tryptophan (trp) is one of the seven essential amino acids necessary for the human body. It is found in relatively low amounts in the body. Unlike other amino acids, the majority of tryptophan circulates in blood mainly bound to albumin (Pardridge 1979). Tryptophan is also the precursor of N-formulkynurenine, which is converted into kynurenine (kyn) by kynurenine formamidase. N-formylkynurenine and kynurenine are the first metabolites of a complex metabolic pathway ending in quinolic acid, niacin, kynurenic and xanturenic acid (Grohmann, Fallarino & Puccetti 2003). Two enzymes, in humans, are able to catalyse conversion of tryptophan into N-formylkynurenine: tryptophan 2,3 dioxygenase (TDO) and Indoleamine 2, 3 dioxygenase (IDO) (Le Floc'h, Otten & Merlot 2011, Luukkainen, Toppila-Salmi 2013). IDO initiates the first and rate-limiting step of tryptophan breakdown along the kynurenine pathway (Grohmann, Fallarino & Puccetti 2003) IDO is expressed in both non-myeloid cells (e.g. epithelial cells, vascular endothelium, tumours) and myeloid cells (e.g. macrophages, DCs, B-cells) (Munn, Mellor 2013). IDO expression is found primarily in mucosal tissues. The IDO pathway is induced in many tissues during inflammation majorly because IDO gene expression is induced by interferons (IFN) (Munn, Mellor 2013, Popov, Schultze 2008, Scheler et al. 2007).

2.6.2.The mechanisms of action of IDO

IDO is able to modify immune responses in several ways. Firstly, IDO produces kynurenine, which acts as a natural ligand for the aryl hydrocarbon receptor (Munn, Mellor 2013). Kynurenine-pathway metabolites act as both immunologically active ligands for the aryl hydrocarbon receptor and as excitatory neurotoxins in central nervous system inflammation (Munn, Mellor 2013). The aryl hydrocarbon receptor is a ligand-activated transcription factor that appears to be

immunosuppressive (Munn, Mellor 2013). It promotes differentiation of Tregs, suppresses anti-tumour responses and decreases the immunogenicity of DCs. The second effect of IDO is the rapid consumption of tryptophan from the local microenvironment (Munn, Mellor 2013). Tryptophan depletion can act as a potent regulatory signal via molecular stress-response pathways e.g. general control nonderepressible 2 (GCN2) kinase, which is a mammalian target of rapamycin (Munn, Mellor 2013). IDO-induced GCN2 pathway seems to enhance Treg activity, whilst inhibiting T effector cells. The shift in favour of Tregs over T cells may control excessive inflammation (Munn, Mellor 2013). Thirdly, IDO has been reported to act as a direct intracellular signalling molecule in DCs expressing it, independently of its enzymatic activity (Munn, Mellor 2013). The metabolic effects of IDO begin as local signals, as it is a non-secreted intracellular enzyme. Neighbouring cells may sense and respond to both secreted kynurenine metabolites and reduced access to tryptophan (Munn, Mellor 2013). In addition, the signalling activity of IDO in DCs turns them into regulatory DCs (Fallarino, Grohmann 2012). DCs can present antigens in an immunogenic or tolerogenic fashion, depending on environmental factors (Grohmann et al. 2003). In certain pathways, antigen-presenting cells and Tregs are involved in an interplay which results in further up-regulation of IDO and also four other amino acid-consuming enzymes, capable of restraining T cell proliferation and promoting Treg expansion (Fallarino, Grohmann 2012, Luukkainen, Toppila-Salmi 2013).

2.6.3. IDO and diseases

2.6.3.1.The role of IDO in airway inflammation

IDO induction in eosinophils might mediate a Th2 polarization, *in vitro* and *in vivo* eosinophils displayed intracellular IDO immunoreactivity (Odemuyiwa et al. 2004). The function of IDO in eosinophils is either stimulatory or inhibitory on Th1 and Th2 cells, depending on the inflammatory model and previous sensitization (Swanson et al. 2004, Grohmann et al. 2007, Luukkainen, Toppila-Salmi 2013). Tryptophan and kynurenine serum concentrations have been found to be higher in seasonal AR patients, in comparison to controls and to be higher out of pollen season than during pollen season (Ciprandi et al. 2010). Moreover, aeroallergen exposure i*n vivo* increased serum IDO activity in asymptomatic atopics compared with either symptomatic atopic or non-atopic individuals (Paveglio et al. 2011). High baseline serum IDO activity associates with better response to immunotherapy (Kositz et al. 2008). Also, signs of decreased IDO activity have been detected after immunotherapy (Kofler et al. 2012).

In patients with CRS, Plasmacytoid- (pDCS) and myeloid DCs were found to be elevated in nasal polyp tissue, in comparison to non-inflamed nasal mucosa. In addition, pDCS were down regulated in more severe cases (e.g. CRSwNP with asthma)(Pezato et al. 2014). IDO levels were also found to be increased in NP, in

comparison to control nasal mucosa, and correlated negatively with the number of pDCS (Pezato et al. 2014).

In patients with asthma, baseline IDO activity in sputum has been shown to be significantly lower than control levels (Maneechotesuwan et al. 2008). Normal baseline activity in asthmatics could be induced by using inhaled corticosteroids (Maneechotesuwan et al. 2008). Similarly, lower IDO activity has been observed in atopics and in allergic asthmatics, in comparison to non-atopics (Maneechotesuwan et al. 2008). Pharmacologic inhibition of IDO worsened symptoms in experimental allergic asthma (van der Sluijs et al. 2013). When exposing *in vitro* monocyte-derived DCs with house dust mite Dermatophagoides pteronyssinus 1, functionally active IDO decreased in cells from patients with house dust mite-sensitive asthma compared to non-atopic asthmatics(Xu et al. 2008b). In murine asthma models, IDO inhibits eosinophilic inflammation, immature DCS expressing IDO improved lung inflammation, eosinophil numbers and lowered cytokine levels (Loughman, Hunstad 2012). Increased CD4+ T cell apoptosis was observed in mice receiving IDO-expressing immature DCs, in comparison to the control groups (Maneechotesuwan et al. 2009).

2.6.3.2. The role of IDO in other diseases

IDO1, IDO2 and TDO have all been shown to associate with cancer phenotype or with inferior prognosis, in several malignancies, such as gastric, colon, renal, breast, and ovarian cancer, acute myeloid leukaemia, malignant gliomas, and melanoma) (Lob et al. 2009, Adams et al. 2012, Ott et al. 2014, Gerlini et al. 2010, Munn et al. 2004, Ryan et al. 2014, Mansfield et al. 200, Theate et al. 2014, Goyne, Cannon 2013, Fukuno et al. 2014, Chen et al. 2014). Sioud et al gave an IDO-silenced DC vaccine to four patients with gynaecological cancers. IDO silencing enhanced the immunogenic function of DCS, both *in vitro and in vivo*, and this was related to objective clinical response (Sioud et al. 2013).

IDO can have opposing roles in host defence against infection. IDO can play a dominant role in directly suppressing pathogen replication (e.g. toxoplasmosis, chlamydial infections, Mycobacteria tuberculosis) (Pfefferkorn 1984, Carlin, Borden & Byrne 1989) or by limiting the spread of virus infection (e.g. human herpes simplex virus type 2, human cytomegalovirus) (Adams et al. 2004, Bodaghi et al. 1999, Obojes et al. 2005, Terajima, Leporati 2005, Schmidt, Schultze 2014). However, disadvantageous effects of IDO have also been reported in viral infections (hepatitis B and C virus, human immunodeficiency virus 1) (Hoshi et al. 2012, Planes, Bahraoui 2013, Boasso et al. 2009, Schmidt, Schultze 2014). Escherichia coli has been found to induce IDO expression in host cells, as a pathogen strategy to promote colonization and establishment of infection (Loughman, Hunstad 2012).

3. AIMS OF THE STUDY

- To evaluate the expression of IDO in the upper airways, in allergic rhinitis and CRS.
- To examine the association of serum IDO activity with asthma and nasal polyps.
- To compare preoperative and postoperative endoscopical findings, computed tomography scans, symptoms and the effect of patient history after simple uncinectomy and uncinectomy with an additional maxillary sinus antrostomy.

4. MATERIALS AND METHODS

4.1. Ethical aspects

Written informed consent was obtained from all patients in each study. Each study was separately approved by the ethical committees of each hospital involved in these studies, respectively, the Ethical board of Tampere University Hospital (decision numbers R07039 (I), R04044 (II), R96032 (III); R01070, R01036, R07038 (IV, V)). Clinical investigation was conducted according to the principles enunciated by the Declaration of Helsinki.

4.2. Study design and population

4.2.1 Study I

This study was carried out at the Department of Otorhinolaryngology, Tampere University Hospital, Finland, from 2003-2010. All the subjects were Caucasian. The inclusion criteria of patients were: diagnosis of CRSsNP, CRSwNP, or ACP based on EPOS criteria of symptoms, endoscopic and sinus computed tomography findings (Fokkens et al. 2012). The exclusion criteria were cystic fibrosis, and diseases with a severe impact on general immunity.

The exclusion criteria of control subjects were: sinonasal disease (except mild allergic rhinitis), or any other disease requiring constant medication. Diagnosis of atopy was based on skin prick test positivity. Diagnosis of asthma was based on clinical features and pulmonary function tests. Diagnosis of acetylsalicylic acid (ASA) intolerance was made on the basis of a history of wheezing or asthma attacks precipitated by non-steroidal anti-inflammatory drugs.

Nasal biopsies were taken from a total of 83 patients (19 healthy volunteers, 54 CRSwNP patients, 10 ACP patients) (figure 1A).

Maxillary sinus biopsies were taken from a total of 67 patients (12 healthy controls, 41 patients with CRSsNP and 14 patients with CRSwNP) (figure 1B).

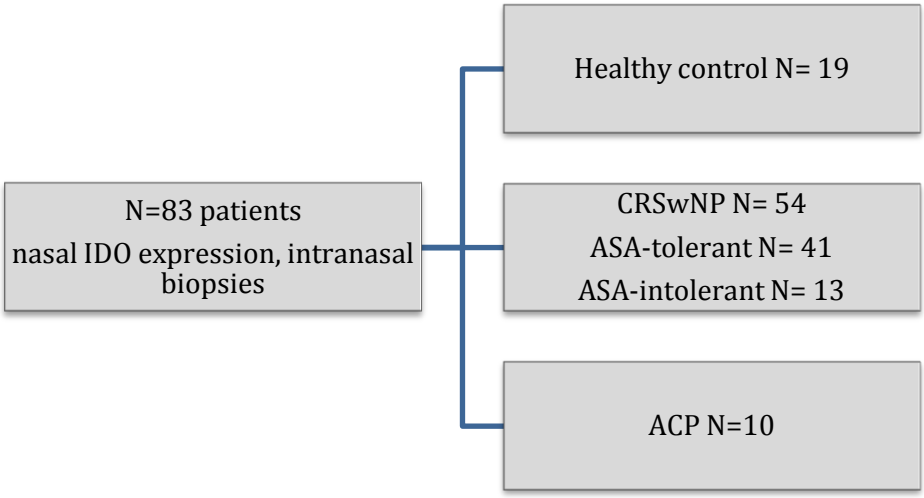

Figure 1A. Study population of study I, nasal biopsies.

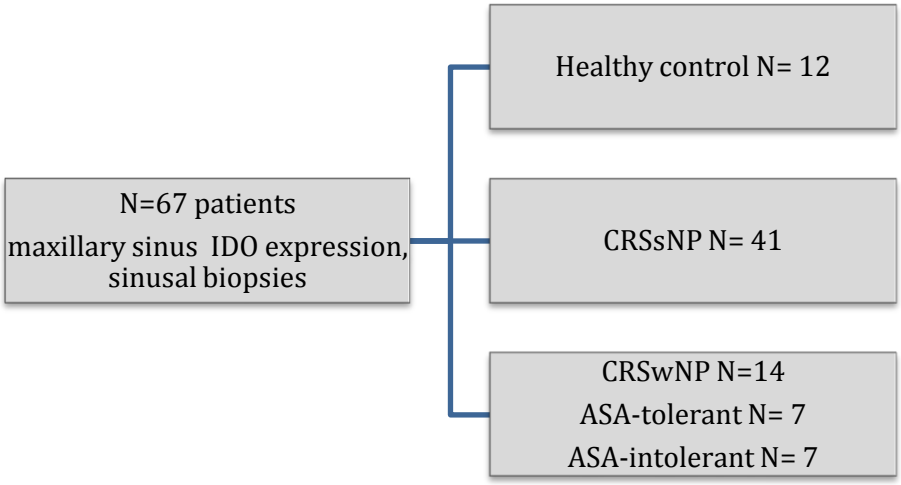

Figure1B. Study population of study I, maxillary sinus biopsies.

4.2.2 Study II

The study was carried out at the Department of Otorhinolaryngology, Tampere University Hospital, Finland from 2005-2011. All subjects were Caucasian, healthy and non-smokers. The atopy group was sensitized to birch pollen and had seasonal birch pollen allergic rhinoconjuctivitis without diagnosis of asthma. The nonatopic control group did not have any disease, were not sensitized to any basic allergens and did not have sinonasal symptoms. Diagnosis of birch pollen-induced allergic rhinitis was based on a history of seasonal allergic rhinitis symptoms during spring, clinical examination, and skin prick test positivity. Patients were not allowed to use their medication (antihistamine and/or nasal corticosteroids) for a minimum of 5 days before nasal biopsies were taken. In addition, absolute eosinophil counts, total blood IgE, specific birch pollen IgE and timothy grass IgE were obtained from all patients. A total of 27 patients were included in this study (15 volunteers, 12 atopics) (figure 2)

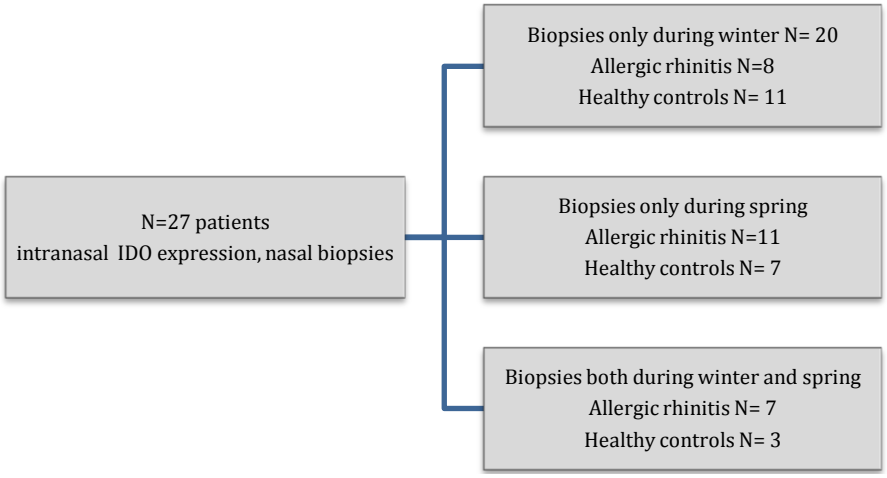

Figure 2. Study population of study II.

4.2.3 Study III

This study was based on previous data obtained in a population-based case-control study of Finnish adult asthma. The study was carried out at the Department of Pulmonology Tampere University Hospital, Finland from 1996-1998, patient interviews taken during 1997 and clinical tests were performed in 1998. Subjects were Caucasian, without diagnosis of cystic fibrosis, and without other diagnoses or medication having a severe impact on general immunity. Inclusion criteria for asthmatic subjects were age over 30 years and entitlement

to special reimbursement for asthma medication from the Social Insurance Institution of Finland. The entitlement is granted if the criteria for persistent asthma are fulfilled, as certified by a chest specialist. Typical history, clinical features, and asthma course must be documented. At least one of the following physiologic criteria is required for diagnosis: (1) a variation of 20% or greater in diurnal PEF recording (reference to maximal value); (2) an increase of 15% or greater in PEF or FEV_1 with β_2 -agonist; or (3) a decrease of 15% or greater in PEF or FEV_1 in exercise testing. Moreover, at least a 6-month period of continuous regular use of anti-asthmatic medication must have elapsed by the time of the decision. One to two control subjects without asthma or chronic obstructive pulmonary disease were initially selected for each subject through a register covering the entire population. No other exclusion criteria were used for control subjects. Patients and control subjects were matched for age, sex, and area of residence. A total of 643 patients were included in this study (figure 3) (Karjalainen et al. 2002, III).

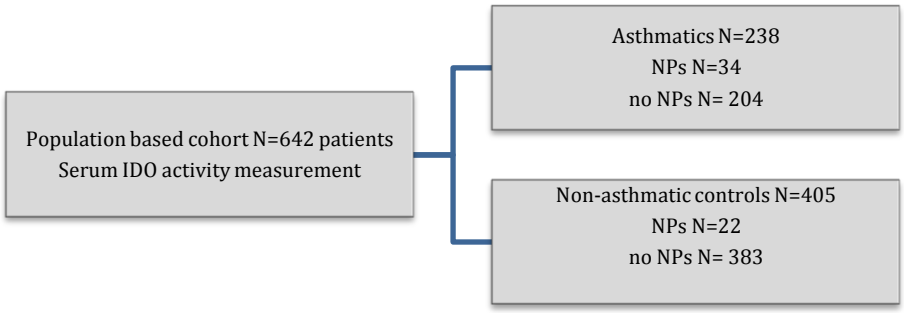

Figure 3. Study population of study III.

4.2.3.1 Determination of atopy and NERD (III)

Atopy was determined by means of skin prick testing performed by specially trained nurses with a panel of 22 common allergen extracts (ALK A/S, Copenhagen, Denmark). These allergens were selected to cover exposures in both urban and rural environments. Skin prick test responses were considered positive if at least one allergen caused a wheal with a diameter at least 3 mm larger than that produced by the negative control. Allergy testing by using the skin prick test method was carried out on 99.1% of asthmatic patients (93 male and 150 female patients) and 99.3% of control subjects (150 male and 252 female subjects) (Karjalainen et al. 2002, III). Both asthmatics and non-

asthmatics were asked if they had other allergic symptoms in addition to skin symptoms: allergic rhinitis, conjunctivitis, and respiratory symptoms. NERD diagnosis was based on the questionnaire. Only asthmatic patients were asked 'Have you ever had wheezing episodes after ingestion of ASA or another non-steroidal anti-inflammatory drug (NSAID), if yes, please specify'. For statistical analysis, patients were classified as having ASA-intolerance if ASA or other NSAIDS caused wheezing.

4.2.3.2 Evaluation of environmental background, NP and asthma severity (III)

The environmental background was evaluated by the following questions: How often were you involved with animals in your childhood? Did you spend your childhood on a farm? Where did you live as a child (urban or countryside environment)? The presence of NP was asked from all patients by the question 'Have nasal polyps ever been found in your nose?' (30). The symptom score of asthma was based on the patients' own subjective assessment on the severity of their asthma: 1=mild to no symptoms, 2=moderate, 3=severe symptoms.

4.2.4 Studies IV, V

The patient population in studies IV and V was the same. The study was carried out in the Departments of Otorhinolaryngology, Tampere University Hospital and Mikkeli Central Hospital, Mikkeli, Finland from 2002-2008. (Figure 4 study population).

The study population was composed of 30 patients with non-polypotic chronic rhinosinusitis. Patients had moderate to severe sinus-related symptoms**,** during at least 12 weeks despite maximal medical treatment (intranasal corticosteroid and/or antihistamine) and a Lund-McKay sinus computed tomography (CT) score (Lund, Kennedy 1995) of at least 6/24 but no more than 18/24.

Following patients were excluded from the study: age less than 18 years; oral corticosteroid treatment during the last two months prior to surgery; previous sinonasal surgery; a history or physical examination suggestive of severe nasal septal deviation (that causes only unilateral nasal obstruction and/or requires septoplasty before FESS can be performed) , unilateral sinusitis, nasal polyposis > grade 1(Lund, Mackay 1993), aspirin sensitivity, chronic bronchitis, CF, a tumour or a disease with a severe impact on general immunity; mild sinus-related symptoms and the following computed tomography findings: severe chronic pansinusitis (total opacification in posterior ethmoidal and/or sphenoidal and/or frontal sinuses and/or total obstruction of the frontal recess).

During follow-up, one patient died accidentally prior to the last postoperative control (9 months postoperatively). Three additional patients missed the last

follow-up (68 months postoperatively), we were unable to contact them by telephone.

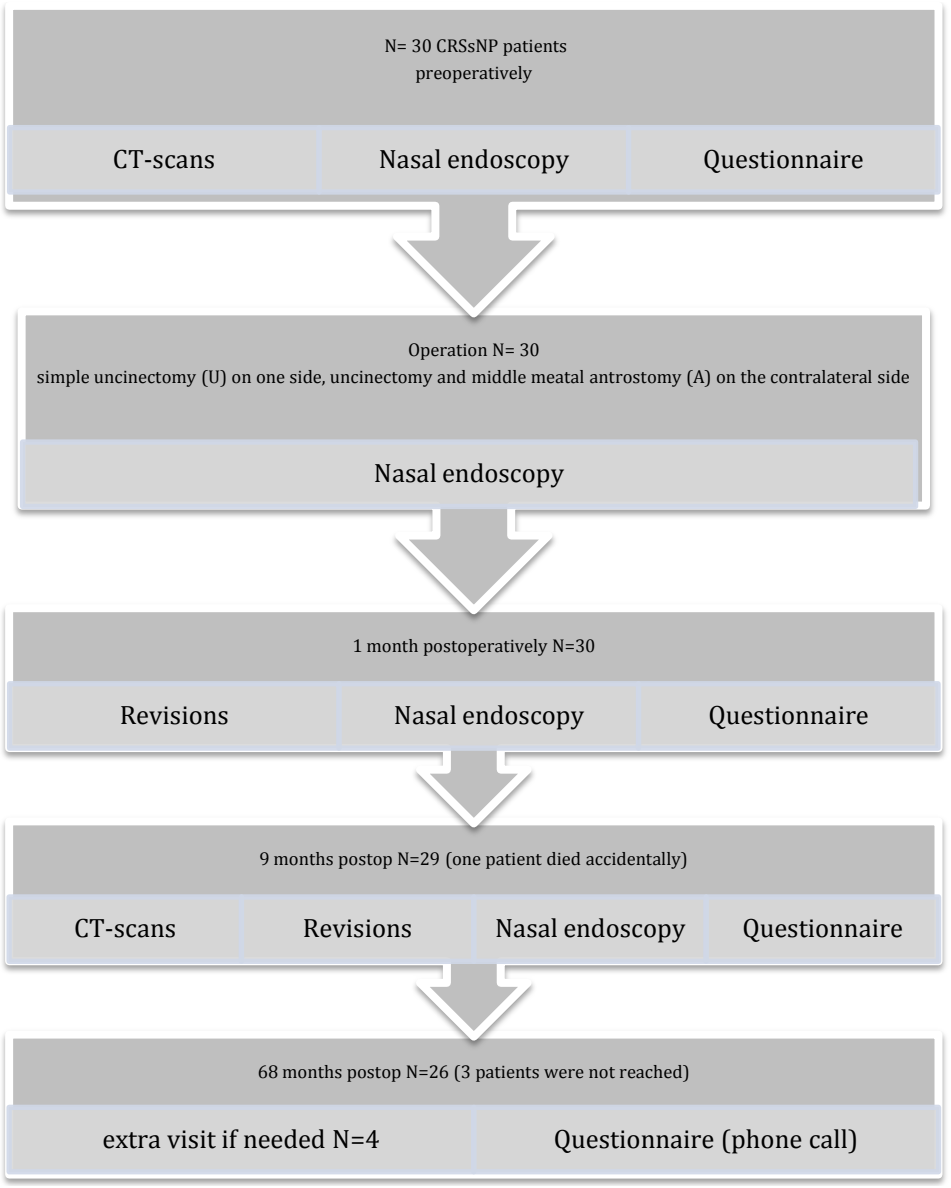

Figure 4. Study population of studies IV, V

postop.= postoperatively Questionnaire includes preoperatively symptoms and postoperatively symptoms, exacerbations and satisfaction with operation technique.

4.3. Laboratory methods

4.3.1 Biopsies

Biopsies were taken from the nasal cavity (I,II) and maxillary sinus mucosa (II). Control nasal mucosa was obtained from the anterior edge of the inferior turbinate, with Fokkens' forceps under local anaesthesia from 18 healthy, non-atopic volunteers who did not use any medication and from 12 AR patients. Biopsies were taken during both winter (off season) and during peak birch pollen allergen exposure in spring (in season, natural allergen exposure) (II, figure 3). 18 of these specimens, taken from healthy non-atopic subjects, were used as healthy controls in study I (Figure 1A). We used nasal polyp specimens from the archives of Fimlab, Laboratories, that had been obtained from patients for diagnostic purposes or undergoing endoscopic polypectomy with or without sinus surgery, under local or general anaesthesia (I).

Maxillary sinus specimens were taken from different individuals than the specimens taken from the nasal cavity. CRS Specimens were obtained during sinus surgery from CRS patients participating in studies IV-V, added by 10 additional CRS patients with or without nasal polyps(Toppila-Salmi et al. 2005). Control maxillary sinus biopsies were obtained from 12 subjects without CRS during orbital decompression or bimaxillary osteotomy (Toppila-Salmi et al. 2005). These patients had never suffered from chronic sinusitis or allergic rhinitis (figure 1B).

4.3.2 Sample staining (I, II)

Nasal specimens, obtained from healthy volunteers (I, II) and patients with allergic rhinitis (II) were immediately snap-frozen in liquid nitrogen and stored at -80°C until analysis. Specimens obtained at the time of surgery (polyp specimens, maxillary sinus mucosa) (I), were formalin-fixed and paraffin-embedded.

For light microscope evaluation, immunoperoxidase staining was used and specimens were cut into 3-5 μm thick frozen sections on Superfrost Plus microscope slides. (Menzel-Gläser, Braunschweig, Germany). Frozen sections were fixed with formalin during 45 minutes. Fully automated immunostaining was performed by Ventana BenchMark LT Automated IHC Stainer (Ventana Medical System, Arizona, USA). Ultraview Universal DAB detection kit (catalogue No. 760-500, Ventana Medical System, Arizona, USA) was used. For epitope retrieval CC1: Tris -EDTA buffer pH 8.0 (catalogue No 950-124,Ventana) was used at 95°C to 100°C for 8 minutes. Endogenous peroxidase was blocked with UV-Inhibitor 3% H202 (Ventana) for 4 minutes at 37°C. Tissue slides were rinsed between steps with Ventana Tris-based Reaction buffer (catalogue No. 950-300, Ventana). Slides were incubated at 37°C for 32

minutes with monoclonal antibody (mAb) anti-Indoleamine 2,3 dioxygenase (1:200, clone MAB5412, Chemicon International Inc., USA) followed by application of Ventana Ultraview HRP Universal Multimer (8 minutes at 37°C). Diaminobenzidine was used as a chromogen and haematoxylin as a nuclear stain. Known positive tissue samples (from coeliac or inflammatory bowel disease) were also used to confirm the staining reliability of all separate staining patches (Wolf et al. 2004, Torres et al. 2007a). The specificity of immunohistochemistry was controlled by omitting the primary antibodies or replacing them with irrelevant antisera.

4.3.3. Light microscopic evaluation (I, II)

Sections were examined with a Leica DM 2000 light microscope (Leica Microsystems GmbH, Wetzlar, Germany) by two independent observers blinded to the experimental conditions. In a sample, there was either moderate or strong immunoreactivity of all epithelial cells or no epithelial positivity at all. Thus, the results were expressed as IDO positive epithelium or IDO negative epithelium. The percentage of IDO positive mucosal leukocytes was counted (I,II). Tissue samples were stained with hemalaun-eosin to calculate the number of mucosal leukocytes and eosinophils /mm^2 (I, II) and to evaluate the inflammation score (II) (0= no inflammation, 1=mild, 2= moderate, 3= severe inflammation). Factors included in the inflammation score were: presence and number of leukocytes and eosinophils, appearance of epithelial cells, subepithelial oedema. Polyp specimens were additionally stained with Periodic acid-Schiff (PAS) for semi-quantitative evaluation of the amount of mucus on polyp surface (I) (PAS+ = little or no mucus, PAS++ = plentiful mucus). PAS classification was evaluated according to grade of magenta on the apical side of epithelium, light magenta meaning little mucus (PAS +) and dark magenta meaning more abundant mucus PAS++).

4.3.4. IDO activity measurement and absolute eosinophil count (III); serum and blood samples

Kyn and trp concentrations in the serum samples for this study have previously been measured by High Performance Liquid Chromatography. Trp was monitored by fluorescence detection at 266 nm excitation wavelength and 366 nm emission wavelength; kyn was measured by ultraviolet absorption at 360 wavelength. Kyn/trp ratio was calculated by relating concentrations of kyn (μmol/l) to trp (mmol/l) allowing an estimate of IDO activity (Karjalainen et al. 2002, Laich et al. 2002).

The eosinophil count was performed using Technicon H3 analysers (Bayer Diagnostica, Tarrytown, NY, USA). Eosinophil numbers are expressed as units in 10^9/l.

4.4. Sinus surgery and clinical evaluation of CRS (IV, V)

4.4.1 FESS

FESS was performed by two study co-auhtors, Jyri Myller and Tommi Torkkeli, under local anesthesia. Uncinectomy was performed on both sides, in which the lower two-thirds of the uncinate
process were removed. Additional middle meatal antrostomy was randomized on the contralateral part of each patient. It was performed by removing with cutting forceps the posterior connective tissue of the natural ostium, to duplicate the diameter. If a large ethmoid bulla was disturbed doing uncinectomy and/or antrostomy, it was opened. Light middle meatal tamponation was removed on the first postoperative day. Nasal endoscopy was performed and the operation area was cleaned during the debridement visit 7-30 days (mean ± SD, 16 ± 5 days) after surgery.

4.4.2. Endoscopy (IV)

The endoscopic evaluation was performed with a rigid endoscope preoperatively, 1-77 days (mean ± SD, 26 ± 23 days) before the operation, perioperatively, and during the debridement follow-up visit at 7-30 days postoperatively, 3 and 9 months postoperatively. Physicians filled a form with the endoscopic findings. The maxillary sinus ostium obstruction was scored: 0= no, 1= yes. The maxillary mucosa was scored: 0= normal (with or without cyst), 1= edema, 2= polypous mucosa. The maxillary mucosal sinus secretions were scored: 0= no, 1= mucus, 2= pus. The endoscopic score of the ostiomeatal complex area was semi-quantitatively determined from the following changes: swollen/polypotic mucosa found in the middle turbinate/anterior ethmoid cells/uncinate process/maxillary sinus ostium/opening of the frontal recess; middle meatal adhesions; and anatomical narrowness of the middle meatus. Endoscopic score 0 was normal , 1=mild, 2=moderate, 3= severe changes of the middle meatus and ostiomeatal complex.

4.4.3 CT- scans (IV)

Coronal sinus CT scans were obtained pre- and 9 months postoperatively. The ostiomeatal complex was reconstructed with 1 mm slice thickness. Lund-MacKay (LM) scores and the area of the ostium were determined**,** The radiological stage of inflammation was determined by two independent observers who did not have knowledge of the patient history or the immunohistochemical results. The stage was based on the appearance on the computed tomography scans according to Lund-MacKay (Lund, Mackay 1993, Lund, Kennedy 1997). Maxillary, anterior ethmoidal, posterior ethmoidal, sphenoidal, and frontal sinuses are graded: 0 = no abnormalities, 1 = partial opacification, 2 = total opacification. For the osteomeatal complex: 0 = not

occluded, 2 = occluded. Total points for both sides was 12 and the radiological stages 0-3 were determined in the following way: 0 points = stage 0, 1-3 points = stage 1, 4-7 points = stage 2, 8-12 points stage 3.

4.4.4. Questionnaires and symptoms reporting (IV, V)

Patients filled the symptom questionnaire 1-77 days (mean ± SD, 26 ± 23 days) preoperatively. The same questionnaire was filled during a control visit to the operating surgeon nine months postoperatively, and later on, based on patients' answers during telephone calls made blindly at 56-86 months (mean ± SD, 68±6.5 months) postoperatively. During the telephone call, if the patient had undergone revision surgery, he/she was asked to answer the questions according to the situation before revision surgery was performed. The following questions were asked preoperatively, and at nine and in average 68 months postoperatively: the number of acute bacterial sinusitis episodes diagnosed or suspected by a doctor during the previous year; and the existence of the following symptoms: facial pain/pressure, nasal obstruction, nasal discharge, postnasal drip, decreased sense of smell (no = 0, mild or moderate = 1, severe = 2). In addition, lacrimation (none = 0, mild =1, moderate = 2, severe = 3) and postoperative bleeding (absent = 0, mild or moderate = 1, severe = 2) were asked during the debridement visit postoperatively and at nine months postoperatively. Satisfaction with the operation was scored according to two questions asked at nine and 68 months postoperatively on each side separately: 'How is the situation in the maxillary sinuses now compared to the situation before the operation' (no symptoms – clearly decreased symptoms – slightly decreased symptoms –no change- more symptoms) and 'If you could choose, would you now be willing for a similar operation?' (yes – maybe –no, reason why if no). The satisfaction was scored in the following way: 0 = good; patient benefited clearly from the operation , 1 = moderate; patient experienced only slight benefit from the operation and is unsure about the willingness for a similar operation if it was performed now, 2 = poor; patient experienced no change or worsening after the operation and is unwilling/unsure for a similar operation.

4.4.5. Evaluation of job exposure (V)

Job exposure was evaluated according to reported current occupation and characterization of work place. The positive job exposure group was determined according to international categorization of high-risk occupations (Kogevinas et al. 2007, Butland et al. 2011). The substances causing job-exposure were: bio aerosols (4 patients), flour (4), mites (3), wood dust (2), reactive chemicals/metalwork (2), moulds (1), and agricultural organic particles

(1). The determination of the patient´s other co-morbidities was based on medical records, interview and medical examination.

4.5. Statistical analysis of results

Data were analysed using Statistical Package for Social Sciences (SPSS Base versions 11.0, 15.0, 16.0, Statistical Software Package, SPSS Inc., Chicago, IL).). Data are expressed as means and medians, when specified (I-III), medians and interquartile ranges (IV-V). Data was always tested to find whether normally distributed or not. The non-parametric Wilcoxon rank sum test (II, IV, V) and McNemar's (II, V) test were used for comparison of matched pairs. The Kruskal-Wallis (I, II,IV) and Mann Whitney U (I-V) tests were used for comparisons of groups. In addition, the t-test (III), Fisher's exact test (I-III) and binary logistic regression (I,III) analyses were used. Results of comparison by binary logistic regression are reported as odds ratios (OR) with 95% confidence intervals. The Spearman rank correlation test was used for correlations (IV,V). A difference was considered to be statistically significant when the two-tailed P-value was less than 0.05. Results were analysed on an intention to treat basis, each patient was analysed according to the randomly allocated treatment (IV-V).

5. RESULTS

5.1 The association of IDO to inflammatory upper airway diseases in sinonasal specimens

The presence of mucosal eosinophils, other leukocytes and IDO was evaluated in control subjects and patients with chronic rhinosinusitis (I) and patients with AR (II) in order to detect IDO protein in upper airway tissues during inflammatory diseases.

5.1.1 Expression of IDO in the nasal cavity during CRSwNP and ACP (I)

IDO was strongly expressed in the vicinity of the Golgi apparatus of epithelial cells, but not on the supraepithelial mucus (table 1, figure 5). IDO was additionally expressed weakly in submucosal leukocytes and intraepithelial glands (table 1, figure 5).In comparison to control inferior turbinate tissue, epithelial IDO positivity significantly associated to CRSwNP and ACP ($p < 0.01$). Moreover, epithelial IDO positivity and a higher number of eosinophils were associated with a high amount of supraepithelial mucus, i.e. strong PAS positivity (respectively $p < 0.05$ and median numbers of mucosal eosinophils 171.2/mm2 and 65.6/mm2 in PAS++ and PAS+ groups respectively, $p < 0.05$). In contrast, the median percentage of IDO positive leukocytes did not significantly associate with the amount of supraepithelial mucus ($p > 0.05$).

	Control	ASA-tolerant CRSwNP	ASA-intolerant CRSwNP	ASA-tolerant ACP	P value
	N=19	N=41	N=13	N=10	
Age					
median	26	59	59	45	<.001*
range	21-62	16-90	22-77	20-79	
No. of male sex	4	28	8	7	.004
Allergic rhinitis	0	8	3	1	.101
Asthma	1	7	12	0	<.001
Smokers	1	10	1	1	.174
Medication					
Antihistamine	0	1	1	0	.087
Intranasal CCS ±antih.	0	18	10	3	<.001
Peroral CCS	0	1	4	0	.006
FESS performed	0	21	5	4	<.001
Recurrent NP	0	4	4	0	.017
No. of eosinophils/mm^2					
median	0.0	131.2	236.0	108.0	<.001*
range	0-32	0-1814.4	14.4-1838.4	0-488	
Specimens with					
epithelial IDO$^+$	16 %	93 %	69 %	70 %	<.001
leukocyte IDO$^+$	26 %	95 %	92 %	80 %	<.001
supraepithelial PAS^{++}	0 %	95 %	92 %	80 %	.107

Table 1 Patient characteristics from biopsies taken from the nasal cavity (I).
Control= mucosa from inferior turbinate from patient without inflammation of nasal mucosa and without sinonasal disease, CCS=corticosteroid, antih.=antihistamine, IDO$^+$ = positivity with mAb anti-indoleamine 2,3 dioxygenase, PAS^{++} = strong Periodic acid shiff –staining positivity, indicating much supraepithelial mucus.

The percentage of IDO positive leukocytes was significantly higher in CRSwNP and ACP specimens, in comparison to control inferior turbinate ($p < 0.001$ and $p < 0.05$, respectively).The median percentage of IDO positive leukocytes correlates with epithelial IDO positivity (median percentages of IDO positive and negative eosinophils 13% and 0%, respectively, $p < 0.001$).

The median number of mucosal eosinophils was higher in CRSwNP and ACP specimens compared to control inferior turbinate ($p < 0.001$). The median number of eosinophils was significantly higher in epithelial IDO positive than in epithelial IDO negative groups (median numbers of mucosal eosinophils 10.4/mm2 and 0.04/ mm2, respectively, $p < 0.001$). Moreover, the number of eosinophils/mm2 correlated significantly with the percentage of IDO positive leukocytes ($p < 0.01$, $r = 0.41$).

5.1.2 Expression of IDO in the maxillary sinus mucosa during CRSsNP and CRSwNP (I)

IDO was expressed in the sinus epithelium and submucosal leukocytes in a similar way as in the specimens taken from the nasal cavity (table 2, figure 5).CRSsNP or CRSwNP groups did not differ in epithelial IDO positivity, in comparison to control sinus mucosa, ($p > 0.05$). In contrast, the median percentage of IDO positive leukocytes was significantly higher in CRSwNP specimens compared to both control sinus mucosa and CRSsNP groups ($p < 0.05$). The number of mucosal eosinophils did not differ in CRSsNP or CRSwNP groups, in comparison to control sinus mucosa ($p > 0.05$). Epithelial IDO positive and negative groups did not differ statistically significantly in the median percentage of IDO positive leukocytes and in the median number of eosinophils (respectively, $p > 0.05$ and $p > 0.05$). The number of eosinophils /mm2 did not correlate with the percentage of IDO positive leukocytes ($p > 0.05$).

	Control	ASA-tolerant CRSsNP	ASA-tolerant CRSsNP	ASA-tolerant CRSwNP	ASA-intolerant CRSwNP	P value
	N=12	N=30	N=11	N=7	N=7	
Age						
median	27.9	45.8	49.8	40.3	56.8	.006*
range	19-56	16- 75	31-64	16-67	30-76	
No. of male sex	4	10	3	6	3	.104
Allergic rhinitis	5	19	6	2	6	.256
Asthma	0	9	4	2	6	.005
Smokers	3	8	1	1	1	.867
Medication						
Antihistamine	0	3	1	1	1	.081
Intranasal CCS± antih.	0	12	4	4	3	.003
Peroral CCS	0	1	0	2	4	<.001
≥1 previous ESS	0	6	4	0	4	.016
No. of eosinophils/mm²						
median	80.0	69.3	176.0	105.0	47.6	.665*
range	0-256.0	0-626.3	10.3-1040.0	0-248.0	0-666.7	
Specimens with						
epithelial IDO⁺	42 %	60 %	27 %	71 %	71 %	.214
leukocyte IDO⁺	33 %	67 %	91 %	86 %	86 %	.027

Table 2 Patient characteristics of specimens taken from the maxillary sinus.
Control = maxillary sinus mucosa from patient without chronic rhinosinusitis, HP = hypertrophic polypoid sinus mucosa, CCS = corticosteroid, antih. =antihistamine, IDO⁺ = positivity with mAb anti-indoleamine 2,3 dioxygenase.

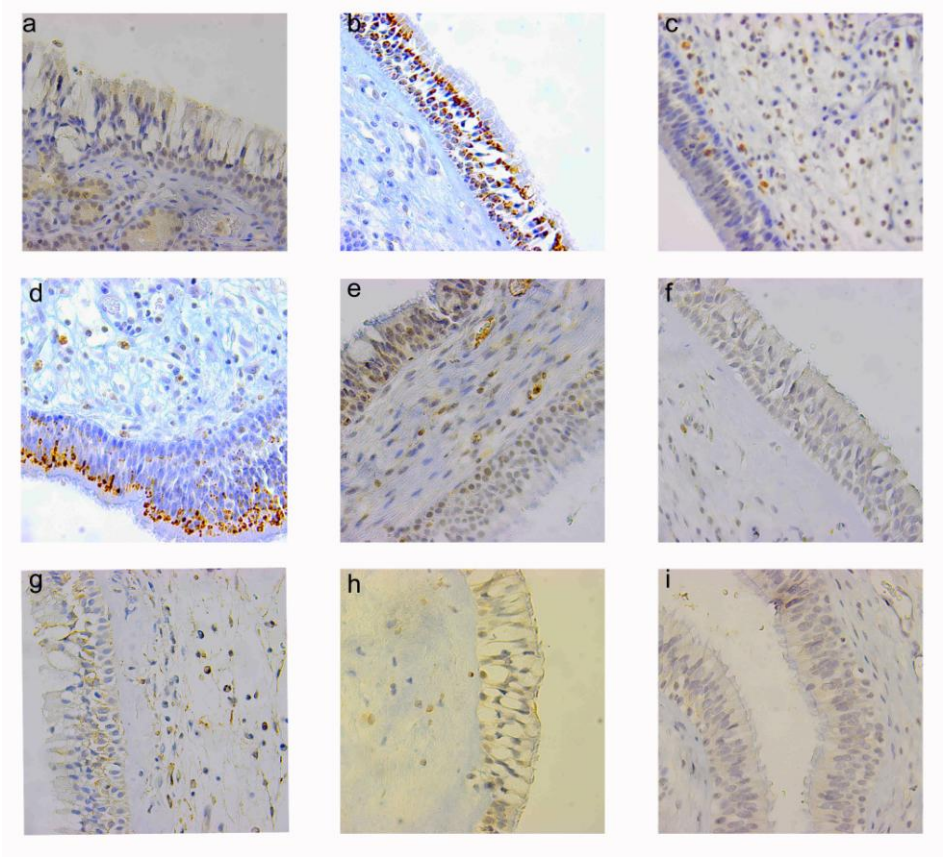

Figure 5 The expression of indoleamine 2,3-dioxygenase (IDO) in biopsies from the nasal cavity (a-d) and maxillary sinus mucosa (e-i).

(a) Control nasal mucosa without epithelial or leukocyte expression of IDO (i.e. epithelial IDO^- and leukocyte IDO^-). (b) Aspirin (ASA) tolerant chronic rhinosinusitis with nasal polyp (ASA-tolerant CRSwNP) with epithelial IDO^+ and leukocyte IDO^-. (c) ASA-intolerant CRSwNP with epithelial IDO^- and leukocyte IDO^+. (d) ASA-tolerant antrochoanal polyp with epithelial IDO^+ and leukocyte IDO^+. (e) Control sinus mucosa with epithelial IDO^- and leukocyte IDO^+. (f) ASA-tolerant CRSsNP with epithelial IDO^- and leukocyte IDO^-. (g) ASA-tolerant CRSsNP with epithelial IDO^- and leukocyte IDO^+. (h) ASA-tolerant CRSwNP with epithelial IDO^- and leukocyte IDO^+. (i) ASA-intolerant CRSwNP with epithelial IDO^- and leukocyte IDO^-. Original magnification 400x.

5.1.3. IDO expression in patients with allergic rhinitis (II)

The control and atopic groups did not differ in terms of patient number, age, sex, peripheral blood eosinophils, percentage of peripheral blood eosinophils. ($p > 0,05$) IDO

was expressed in the vicinity of the Golgi apparatus of epithelial cells, but not on the supraepithelial mucus. IDO was additionally expressed weakly in

submucosal leukocytes and in intraepithelial glands. When observing the nasal biopsies, the number of specimens having positive epithelial IDO staining was not associated with atopy during either winter or spring ($P>0.05$, Table 3, Figure 6). The percentage of IDO positive leukocytes did not associate with atopy in specimens taken during either winter or spring ($P>0.05$, Figure 6). Nor did it associate with the expression of IDO in the epithelium ($P>0.05$,). The subjects did not have changes in the epithelial IDO expression or in the percentage of IDO positive leukocytes when comparing specimens taken during winter and spring from the same individuals ($P>0.05$). The median number of mucosal eosinophils was significantly higher in atopic than in non-atopic subjects only during symptomatic spring ($P=0.044$), whereas the percentage of eosinophils did not significantly differ between atopic and non-atopic subjects ($P>0.05$). Interestingly, during spring the number of mucosal leukocytes and the percentage of IDO positive leukocytes correlated significantly($P<0.05$, $r=0.46$).

	Control N=15	Atopy N=12	P value
Age			
median	23	24.5	NS
min-max	21-36	21-34	
No. of male sex	4	5	NS
Peripheral blood eosinophils			
median	0.23	0.15	NS
Q1-Q3	0.085-0.295	0.12-0.20	
% of peripheral blood eosinophils			
median	3.0	2.5	NS
Q1-Q3	2.0-4.0	2.0-4.0	
S-IgE			
median	27.0	182.0	<.001
Q1-Q3	18.5-58.5	67.0-410.0	
IgE birch			
median	<.35	62.5	<.001
Q1-Q3	<.35-<.35	12.0-161.0	
IgE timothy grass			
median	<.35	4.75	<.001
Q1-Q3	<.35-<.35	1.6-.6.1	
No. subjects having SPT			
positivity to any basic allergen	0	12	<.001
birch	0	12	<.001
timothy grass	0	9	<.001
other pollen	0	12	<.001
animal dander	0	9	<.001
house dust mite	0	0	NS
other basic allergens	0	0	NS
Biopsies were taken during			
winter	11	8	NS
spring	7	11	.019
both	3	7	NS

Table 3. Patient characteristics (II).
Age expressed in years. S-IgE= serum IgE.; Q1= first quartile; Q3= third quartile. SPT= skin prick test.

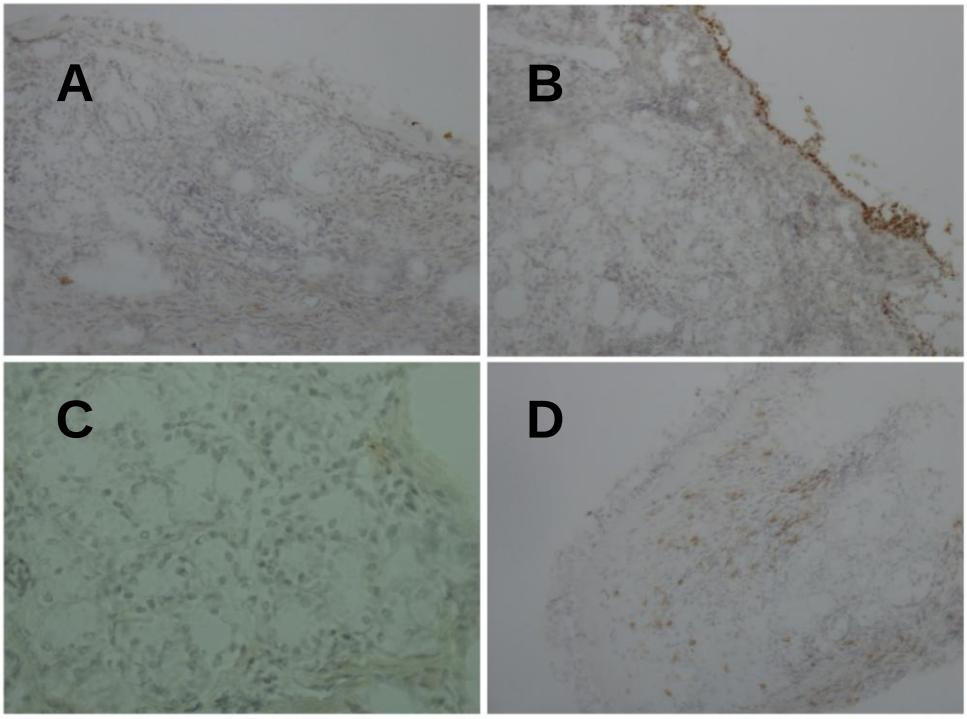

Figure 6 The expression of indoleamine 2,3-dioxygenase (IDO) in biopsies from the nasal cavity. Original magnification 200x (A, B, D) and 400x (C).
(A) Nasal mucosa taken from a patient with allergic rhinitis off-season . No epithelial or leukocyte expression of IDO.
(B) Specimen from another patient with allergic rhinitis, taken off-season, has epithelial IDO^+ and leukocyte IDO^-.
(C) The same allergic rhinitis patient as in frame A, did not display any IDO expression in the specimen taken in-season.
(D) Specimen from a healthy patient, taken off-season, shows epithelial IDO^- and leukocyte IDO^+.

5.2. Relationships of IDO activity and cofactors with asthma and nasal polyps (III)

IDO activity, measured by the serum kynurenine to tryptophan ratio, was studied in asthma, CRSwNP, and atopy, in order to detect alterations of IDO activity on serum level during airway inflammation. Moreover the relationships between blood eosinophilia and childhood environment on asthma, CRSwNP and atopy were evaluated. Table presents the study population.

	Control N=405			Asthma N=238		
	sNP N= 383	wNP N=22	P-value	sNP wNP N=204	N=34	P-value
Age (mean, y)	60	60	.98	59	58	.47
Range	31-89	36-80		31-84	35-76	
Gender male (N)	37% (140)	50% (11)	.26	39% (79)	41% (14)	.85
Never smoked (N)	59% (227)	45% (10)	.27	52% (106)	50% (17)	.34
Ex-smokers (N)	21% (80)	27% (6)		34% (70)	29% (10)	
Smokers (N)	19% (73)	27% (6)		17% (34)	24% (8)	
Atopy (N)	37% (141)	59% (13)	.05	59% (121)	47% (16)	.27
ASA-intolerance (N)	-	-	-	12% (24)	26% (9)	.03
Duration of asthma mean,y (SD)	-	-	-	11.3 (12.1)	11.7 (8.5)	.09
Symptom score median (SD)	-	-	-	2 (1.0)	2 (0.9)	.67
FEV1-percentage mean (SD)	102.0 (19.6)	111.9 (19.9)		86.2 (24.0)	87.7 (25.6)	.86
B-Eos mean, 10^9/l (SD)	0.13 (0.10)	0.18 (0.12)	.05	0.20 (0.15)	0.27 (0.23)	.08
S-kyn/trp mean (SD)	27.6 (9.8)	23.6 (7.3)	.05	27.1 (9.1)	24.2 (8.0)	.05
Childhood environment on farm	39 %	27 %	.37	37 %	31 %	.70
Childhood environment in countryside	74 %	77 %	.81	65 %	66 %	1.00

Table 4 Patient characteristics (III)

Y= age in years; B-eos= blood eosinophilia; SD= standard deviation.

5.2.1 IDO activity

643 patients were enrolled in this study. Patient characteristics are shown in Table 2. Serum IDO activity did not associate with asthma or reported aspirin intolerance (OR=0.993, 95%CI=0.98-1.01, P=0.406; respectively Table 5). Nor did IDO activity correlate with the FEV1-percentage value, duration and symptom score of asthma (P>0.05). However, in contrast to this, low serum IDO activity was associated with patient-reported doctor-diagnosed CRSwNP (OR=0.946, 95% CI = 0.91-0.98, P=0.006; table 5). The result remained the same, when adjusted to asthma, smoking, atopy, and ASA intolerance. On the other hand, low IDO activity was associated with atopy, (OR=0.973, 95% CI = 0.96-0.99, P=0.005, tables 2 and 3, (27).

	NP			Atopy		
	OR	95% CI	P-value	OR	95% CI	P-value
IDO activity	0.95	0.91-0.98	.01	0.97	0.96-0.99	.01
B-eos	1.19	1.05-1.34	.01	1.10	0.99-1.21	.08
NP	-			1.34	0.77-2.32	.30
Farm	0.69	0.42-1.13	.21	0.65	0.47-0.90	.01
Countryside	0.98	0.59-1.61	.94	0.66	0.47-0.92	.01
	Asthma			ASA intolerance		
	OR	95% CI	P-value	OR	95% CI	P-value
IDO activity	0.99	0.98-1.01	.41	0.97	0.93-1.02	.22
B-eos	1.31	1.16-1.48	<.01	1.18	0.98-1.42	.09
NP	2.90	1.66-5.08	<.001	2.70	1.13-6.46	.03
Farm	0.90	0.68-1.18	.51	1.36	0.73-2.55	.42
Countryside	0.67	0.47-0.94	.02	1.22	0.63-2.38	.63

Table 5 The associations between serum IDO activity and NP, atopy, asthma and ASA intolerance as well as the number of peripheral blood eosinophils (B-eos), NP, the farm and the countryside environments.

B-eos values are expressed in 10^9/l units. OR= Odds Ratio; 95%CI= 95% confidence intervals.

5.2.2.Blood eosinophilia

Increased blood eosinophils were associated with asthma (table 5). The association was found only if the patient was nonatopic, or had never smoked (data not shown). In addition, elevated blood eosinophils were observed during

patient-reported doctor-diagnosed CRSwNP (table 5). The association was found only if the patient was nonatopic, or had concomitantly asthma, or had never smoked (data not shown). Blood eosinophilia was not associated with atopy or aspirin intolerance (table 5). This result remained the same when adjusted to NP, atopy, ASA intolerance and smoking (data not shown).

5.2.3. Relationships between asthma, CRSwNP and aspirin intolerance

14.3 % of asthma patients and 5.4 % of controls reported having doctor-diagnosed NP, which differed significantly (P<0.001). Atopy or smoking did not associate to NP (table 5).

33 (13.9 %) out of 238 asthma patients had patient-reported aspirin intolerance. 9 (27 %) aspirin intolerant asthmatics had concomitantly patient-reported doctor-diagnosed NP. Thus, the prevalence of NP in aspirin sensitive asthma was 27 %, and in aspirin-tolerant asthma 12 % (P=0.031). Similarly, asthma patients with NP reported more frequently coexisting aspirin intolerance than asthma patients without NP (P=0.031, Table 1).

5.2.4.Effect of rural environment during childhood

Both childhood spent on the farm or in the countryside protected from atopy (Table 5).

Farm environment during childhood did not associate with asthma, but the countryside environment had a protective effect (Table 5). The protective effect was not observed, if the patients were not atopic [OR= 1.00; 95% CI (0.59-1.70; P= 0.99)]. Childhood spent on the farm or in the countryside did not associate with patient-reported doctor-diagnosed CRSwNP (Table 2). The result remained the same when adjusted by atopy or asthma (data not shown).

5.3. Outcomes of endoscopic sinus surgery in patients with CRSsNP (IV, V)

Endoscopic (IV) and subjective (V) outcomes were evaluated in patients with CRSsNP, each patient underwent endoscopic maxillary sinus surgery with the ostium-preserving technique on one side and the ostium-enlarging technique on the contralateral side.

Characteristics of patients	preop	9 mo postop	68 mo postop
Age at the operation, years			
median	50	54	56
Range	21-66	22-66	27-72
No. of male sex	11	10	8
Allergic rhinitis	16	16	14
Asthma	10	10	10
No. of patients with job exposure.	15	15	12
Nasal polyps	0	1	1
Smokers	7	7	7
Medication			
Antihistamine	1	2	3
Intranasal CCS ±antihistamine	11	10	18

Table 6 Patients characteristics (IV,V)
Intranasal CCS= intranasal corticosteroids.

5.3.1. Endoscopic outcomes (IV)

We used the endoscopic score to evaluate semiquantitatively the mucosal status of the operated area. A high endoscopic score indicated swollen or polypous mucosa and/or anatomical narrowness of middle meatal and ostiomeatal complex area.

Preoperative observation of both sides of each CRS patient revealed no significant differences statistically in the middle meatal endoscopic scores ($p>0.05$, Figure 7). There were no peri- or postoperative differences between the operation techniques in terms of accessory ostium, maxillary mucosal edema and secretions, endoscopically evaluated ($p>0.05$, $p>0.05$ and $p>0.05$, respectively). Significant and identical improvement on both the ostium preserving and enlarging sides was observed, when comparing the preoperative endoscopy scores to 9 months postoperative endoscopic scores of each side

separately, a (p=0.004, p=0.001, respectively, Figure 7), as expected. The endoscopic score was better on the antrostomy side compared to the uncinectomy-only side, but only at 16 days postoperatively (p =0.039, Figure 7).

At 16 days postoperatively, eight obstructed maxillary sinus ostia were found on the uncinectomy-only side in contrast to only one on the antrostomy side (p=0.031). At 9 months postoperatively, five new ostium obstructions were identified, now with a total of 6 on the uncinectomy side and four on the antrostomy side(p >0.05).
At 9 months postoperatively, we identified the following statistically insignificant trends. Maxillary sinus mucosal edema was more frequently found on the uncinectomy-only side compared to the antrostomy side (P=0.083). Furthermore, at 9 months postoperatively four patients had mucous secretions in the maxillary sinus, of which three on the uncinectomy-side. None of the patients had pus or polypous mucosa in the maxillary sinus. However, there was a correlation between maxillary sinus secretions and endoscopic middle meatal score at 9 months postoperatively, but not with maxillary mucosal eodema (the uncinectomy-only side P<0.01, R=0.67; the antrostomy side P<0.01, R=0.58, p>0.05, respectively).

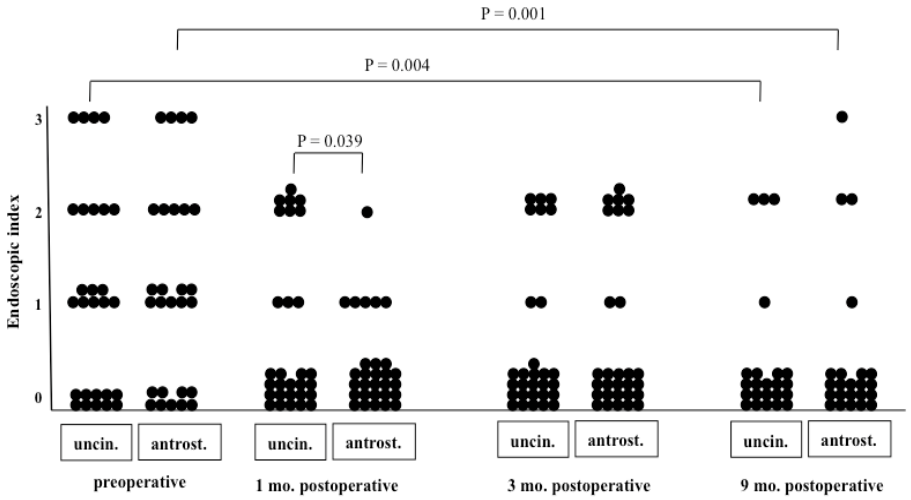

Figure 7 The endoscopic index of the middle meatus at different time points
The endoscopic index on the y-axis (0= normal, 1=mild, 2=moderate, 3= severe changes) was semi-quantitatively determined. The following changes were taken into account: swollen/polypotic mucosa found in middle turbinate/anterior ethmoid cells/uncinate process/maxillary sinus ostium/opening of the frontal recess; middle meatal adhesions; and other kind of narrowness of the middle meatus.

5.3.1.1 Correlation of endoscopic findings to paranasal CT-scans

Interestingly, at 9 months postoperatively there was a correlation between the endoscopic score and the radiologic maxillary sinus LM score (the uncinectomy-only side $p<0.01$, $R=0.63$; the antrostomy side $p<0.05$, $R=0.51$). Preoperatively there were no correlations between these variables ($p<0.05$).

The ostium findings by CT-scans and by endoscopy correlated at 9 months postoperatively, to the exception of one case; the median of radiologic ostium area was higher in cases with endoscopically patent ostium contrasted to those with an endoscopically obstructed ostium (the uncinectomy-only side $p=0.003$, the antrostomy side $p=0.009$).

At nine months postoperatively, endoscopically evaluated maxillary mucosal edema correlated with the radiologic maxillary sinus LM score on both uncinectomy and antrostomy sides ($P<0.01$, $R=0.77$; $P<0.05$, $R=0.46$, respectively).

5.3.1.2. Correlation of endoscopic findings to patient factors, symptoms and adhesions

Allergic rhinitis associated with a higher endoscopic middle meatal score, but only on the ostium-enlarging side and at 9 months postoperatively ($p=0.037$). In contrast, the endoscopic score did not associate to age, sex, smoking, asthma, or medication ($p>0.05$).

Sex, allergic rhinitis, asthma, smoking, nasal corticosteroids and/or antihistamine use did not associate with an obstructed maxillary sinus ostium, a swollen maxillary sinus mucosa or secretions or adhesion formation (asthma not tested) (respectively $p>0.05$, $p>0.05$ and $p>0.05$).

Pre- or postoperative endoscopic scores correlate with any of the symptoms asked at the same time points on either ostium-preserving or –enlarging sides ($p>0.05$).

Maxillary sinus obstruction did not associate to any of the asked symptoms at 9 months postoperatively ($p>0.05$). A swollen maxillary sinus mucosa, the presence of adhesions or secretions did not associate with any of the symptoms asked postoperatively at the same time points on either ostium-preserving or –enlarging sides ($P<0.05$ and $p>0.05$, respectively).

Preoperatively, there were no signs of adhesions. At 9 months postoperatively six patients out of 29 had endoscopic findings of adhesion formation; three were on the uncinectomy side and four on the antrostomy side. There were no statistically significant differences between sides in terms of adhesions ($p>0.05$).

5.3.2. Subjective outcomes (V)

Patient symptoms and satisfaction after maxillary sinus surgery with the ostium-enlarging and –preserving techniques were evaluated at several time points postoperatively. In addition, we took into account patient history, rate of exacerbations and the need for revision sinus surgery when reviviewing the helpfulness of surgery in CRSsNP management .

5.3.2.1. Symptoms during the debridement visit

During the debridement visit at 7-30 days (mean ± SD, 16 ± 5 days) postoperatively, the patients were asked about symptoms during immediate postoperative recovery: pain, bleeding, lacrimation and nasal obstruction. There were no significant differences between the operation techniques in the median values of each of these four symptoms ($p>0.05$). The median sum of these four symptoms as well as the median points of pain, obstruction and bleeding decreased on both sides between the debridement visit and a visit at nine months postoperatively, indicating good recovery on both sides ($p<0.001$).

5.3.2.2. Post-operative symptoms (9 and 68 months post-surgery)

When comparing preoperative and postoperative (9 months and 68 months) symptoms: facial pain , nasal obstruction, and discharge values and the mean of these three values, a significant reduction on both the ostium preserving and enlarging sides was observed ($p<0.001$, Figure 8A). There was no significant difference between operation techniques in these values ($p>0.05$, Figure 8A). Moreover the delta values indicating the change of these three symptoms before and after the operation, did not differ between the operation techniques at nine months and in average 68 months postoperatively values ($p>0.05$). Symptom values for reduced sense of smell and postnasal drip could not be compared between the sides, however they declined significantly when comparing preoperative and postoperative (nine months and 68 months) values ($p<0.001$).

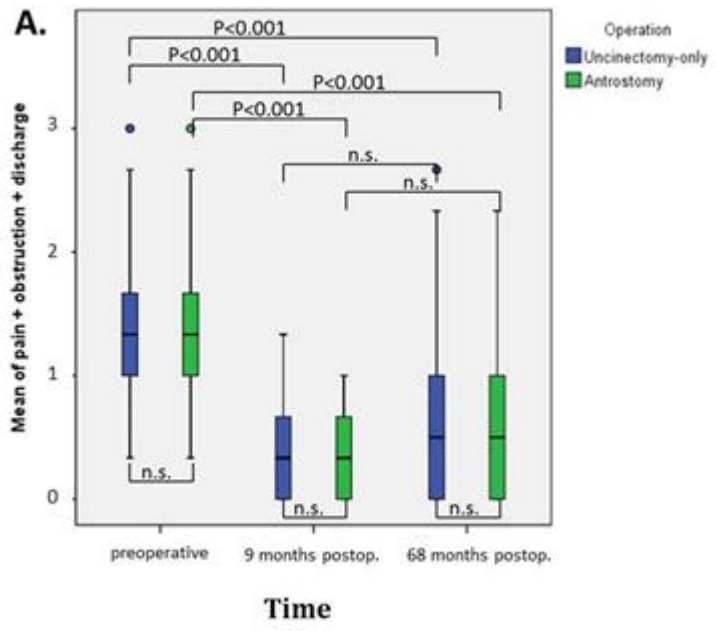

Figure 8A Comparisons of mean value of pain, obstruction and discharge scores between operation techniques at different time points

n.s.= not significant. Horizontal lines represent medians; upper and lower vertical bars represent the 75th and 25th percentile ranges; vertical lines represent the 99th percentile range. The Y-axis represents the combined mean values of pain, obstruction and discharge with the following values 0=no, 1=mild, 2=moderate, 3=severe.

5.3.2.3. Satisfaction with operation and revision surgery

When observing satisfaction with the operation at nine months and on average 68 months postoperatively, the majority of patients expressed good/moderate satisfaction and there were no differences between operative techniques in the reported satisfaction ($p>0.05$). Revision surgery was performed on one antrostomy side and three uncinectomy-only sides for 3 out of 26 patients during the observation period, however this difference between the sides remained statistically insignificant ($p>0.05$). The two patients (one male and one female) that underwent revision surgery only on the uncinectomy side, had complaints solely on this side before revision antrostomy was performed. Of the 3 patients that underwent revision surgery, all were non-smokers, had allergic rhinitis but not asthma. The patient with bilateral revision surgery had additionally job exposure.

5.3.2.4. Exacerbations of CRSsNP post-surgery

The exacerbation-rate could not be compared between sides. When comparing preoperative and postoperative (nine months and 68 months) exacerbation-rates, e.g. the numbers of reported antibiotic courses for doctor-diagnosed sinusitis during the last year, the number decreased significantly (figure 8B). Interestingly, the number of acute sinusitis per year increased significantly between 9 and 68 months postoperatively (figure 8B).

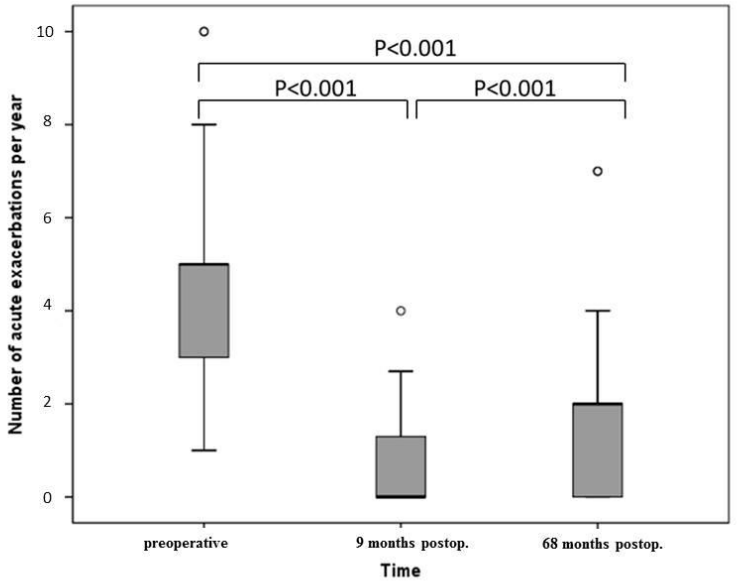

Figure 8B The patient-reported number of acute exacerbations e.g. prescribed antibiotic courses for doctor-diagnosed sinusitis, per year.

Preoperatively, at 9 and 68 months postoperatively, patients reported the number of antibiotics prescribed. Horizontal lines represent medians; upper and lower vertical bars represent the 75th and 25th percentile ranges; vertical lines represent the 99th percentile range. Values in the y-axis represent actual prescribed antibiotic numbers.

For analysis and figure 8B, the number of antibiotics prescribed during the postoperative follow-up time period (9 or 68 months), for the 9-month period the value was multiplied by 12/9=1.33.

5.3.2.5. The influence of patient history

When analysing the median values of pre- or postoperative symptoms and satisfaction for either ostium-preserving or –enlarging sides, there was no association to sex, allergic rhinitis and/or asthma, smoking, job exposure, or

intranasal corticosteroid and/or antihistamine medication ($p>0.05$). Moreover, these symptom and satisfaction values did not correlate with age or the number of acute sinusitis/year ($p>0.05$) . Interestingly, there was a trend that patients with asthma and/or job exposure expressed more frequently satisfaction only on the side with antrostomy, or neither technique provided them satisfaction ($P=0.054$, Figure 9). The unsatisfied patients had the possibility to come for an extra control visit with nasal endoscopy at in average 68 months postoperatively (Figure 4).

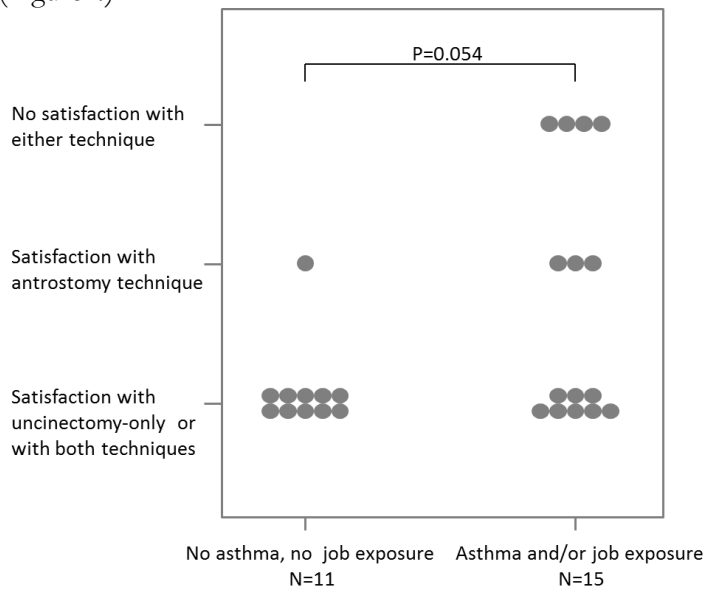

Figure 9 Comparison of the operation-technique with which the patient experienced greater satisfaction on average at 68 months postoperatively.
Each patient is represented by a dot.

6. DISCUSSION

Susceptibility to chronic airways inflammation is currently thought to develop in individuals with distinct epigenetic and genetic elements. So far, these elements are not very well characterized and they are in interaction with various environmental factors (Busse 2011). The early development of barriers and immunity together with environmental exposures that occur throughout life, are thought to contribute importantly to the pathogenesis of chronic inflammatory diseases (Renz, Brandtzaeg & Hornef 2011). Environmental factors affect the host organism as a whole. Host genetics are modified by current and even past environmental factors (Pembrey et al. 2014, Franklin et al. 2010). Upper and lower airways form a united airway. Processes in both anatomic parts can affect each other and occur concomitantly in the whole respiratory tract.

We demonstrated that IDO expression is pronounced in the nasal mucosa of CRSwNP and ACP patients and correlates with higher amounts of supraepithelial mucus and mucosal eosinophils. IDO expression levels in leukocytes were higher in maxillary sinus mucosa biopsies from CRSwNP patients, when compared to CRSsNP and healthy subjects. Elevated IDO expression has been shown to indicate the presence of certain human viral infections, including Human immunodeficiency virus, hepatitis B and C viruses or influenza, as well as major bacterial infections such as tuberculosis, listeriosis and bacterial sepsis (Schmidt, Schultze 2014). IDO has infection-limiting as well as promoting roles, depending on pathogen (Schmidt, Schultze 2014). Bearing this in mind, it is interesting that we found similar IDO expression in the maxillary sinus epithelium of healthy controls and CRS patients. IDO expression in the maxillary sinus epithelium could have importance in limiting bacterial colonization, promoting tolerance to inhaled foreign particules or on the other hand could indicate presence of atypical bacterial colonization. The significance of IDO expression in the maxillary sinus epithelium remains unsolved.

We found lower serum IDO activity in patients with CRSwNP, independently of asthma, atopy, ASA intolerance and smoking. Others have also shown that the *INDO* genotype (found in nasal polyp specimens) seems to have a role in the genetic risk for aspirin intolerant asthma (Sekigawa et al. 2009). NP might thus affect immunity on a systemic level independently of asthma, atopy or ASA-intolerance. Patients with CRSwNP might have a high level of epithelial IDO in the nasal mucosa, but low serum activity of IDO requires further elucidation. The explanation could partly be that active epithelial IDO does not affect or affects serum kyn/trp concentrations little. Local and systemic IDO probably have different immunoregulatory functions in patients with CRSwNP (Luukkainen, Toppila-Salmi 2013). IDO

expression may allow aberrant immune responses to go on locally, and possibly systemically in CRSwNP, which could contribute to epithelial remodelling

Nasal biopsies fom subjects with AR demonstrated that the expression of IDO remained at a low level even in spring, when atopic subjects showed increased mucosal eosinophilia and symptom score. Among CRS patients, expression of IDO was independent of the atopic status of the patients. Thus, in the upper airways, IDO seems to associate with CRSwNP but not with allergic rhinitis (Luukkainen, Toppila-Salmi 2013). This might mean that IDO may not have a local role in pollen AR in the nasal mucosa.

We were not able to show that serum IDO activity associates with asthma, its severity, or reported aspirin intolerance (III). In contrast to our findings, that showed that local IDO does not associate with pollen AR and on a systemic level with asthma, other studies propose that IDO has a role in the control of atopic airway diseases (Odemuyiwa et al. 2004, von Bubnoff, Bieber 2012). Pharmacologic inhibition of IDO worsened symptoms in experimental allergic asthma (Hayashi et al. 2004). A study with IDO knockout (IDO -/-) mice did not reveal a role for IDO in antigen-induced immune tolerance in the airways but instead, IDO seemed to promote antigen driven Th2 responses via effects on lung DCs (Xu et al. 2008b). The controversy might be explained in part by the fact, that previous studies have mostly addressed animal and cell models of atopic asthma or allergy. On the other hand, in our study, we observed asthma in adult patients that was not clustered into phenotypes (e.g. atopic asthma, non-eosinophilic asthma). It is possible that had we identified phenotypes of asthma, an association between IDO activity and a certain phenotype of asthma could have been found. Similarly, it would have been of interest to study systemic IDO activity in allergic rhinitis before and after allergen exposure.

In addition to atopic asthma, previous studies have demonstrated a high serum IDO activity to be associated with immunosuppression in pathogenic tumorigenesis, renal insufficiency, pulmonary tuberculosis, coeliac disease and community acquired pneumonia (Vasilyeva et al. 2012, Suzuki et al. 2012, Suzuki et al. 2011, Outinen et al. 2011, Torres et al. 2007b). Comparing respiratory tract inflammations to allografts, inflammatory bowel disease and cancer may be far-fetched. In allografts, IDO-mediated T-cell inhibition has been shown to be rapidly reversed once IDO activity ceases (Hainz, Jurgens & Heitger 2007). Pharmacologic inhibition of IDO causes marked exacerbation of inflammation and worsened symptoms of disease in a murine model of inflammatory bowel disease (Gurtner et al. 2003). In cancer, IDO seems to decrease recruitment of antitumour immune cells, induce tolerance towards tumour antigens and thus facilitate immune escape. In addition, increased IDO

expression correlates with diverse tumour progression parameters and shorter patient survival (Munn, Mellor 2007). Immunologic tolerance to tumours is not simply a passive event; the immune system, in some cases, seems to be aware of tumour antigens but is somehow rendered tolerant to them. Once this acquired tolerance has been established, immunization with tumour-associated antigens can intensify antigen-specific immunosuppression (Munn, Mellor 2007, Luukkainen, Toppila-Salmi 2013). However, inflammatory airway diseases have pathomechanisms that resemble in part the pathomechanisms in immunosuppression in allografts, inflammatory bowel disease, infections and cancer. These shared pathomechanisms might be immune escape, mediation of tolerance, presence of microbial infections in airway mucosa and similarities between the gastrointestinal tract and airway mucosa. It might also be possible that in the airway mucosa, IDO does not have the same roles as in other conditions such as in cancer or inflammatory bowel disease.

Asthma and CRS, especially the CRSwNP subgroup in CRS, are diseases characterized by epithelial remodeling and modification of physiology at the same time or as a consequence. Removal of eodematous obstructive mucosa might normalize maxillary sinus function in CRS. Endoscopic findings, such as the size of the middle turbinate, possible adhesions, crusts and inflammation, have shown to have acceptable interexaminer reproducibility and are suitable for evaluating ESS (Smith et al. 2012, Nair 2009). We found better early recovery of the middle meatal mucosa and maxillary sinus ostium patency on the side with an additional antrostomy. In the long-term, the recovery of the middle meatal mucosa was similarly achieved with both procedures. Similarly to our findings, a randomized controlled study showed that antrostomy was better than uncinectomy alone, in short-term follow-up but that at one year postoperatively a 60% rate of patency was achieved with both procedures (Wadwongtham, Aeumjaturapat 2003). Supporting our findings, in a prospective randomized controlled study, no differences were found in subjective outcomes after performing a large (> 16 mm) or small (< 16 mm) middle meatal antrostomy (Albu, Tomescu 2004, Catalano 2004, Wadwongtham, Aeumjaturapat 2003). Furthermore, our findings of a successful long-term endoscopical recovery with either technique are in correlation with the observations of others (Mace et al. 2010, Guo et al. 2010). Our study demonstrated that half of early postoperatively obstructed ostia remained obstructed later on with other signs of poor recovery. This finding is in accordance with a study showing that successful recovery at only one month postoperatively seems to predict also long-term success of ESS (Rudmik et al. 2011). On the other hand, previous observations have shown that postoperative mucosal healing takes more than one month (Huang et al. 2005, Watelet et al. 2004, Watelet et al. 2002, Xu et al. 2008a). Endoscopic findings did not associate with symptoms in our study, which is in accordance with other

observations (Mace et al. 2010, Ryan, Ramachandra & Hwang 2011, Bradley, Kountakis 2005).

Our study demonstrated that ESS reduced significantly all asked sinonasal symptoms. The reduction of symptoms that the patient was able to compare between sides (facial pain, nasal obstruction, and discharge) were similar with both surgical techniques. A study compared ESS to continued conservative therapy in patients with refractory CRS. The study concluded ESS to be more effective than conservative therapy in improving QoL (measured by sinonasal outcome test-22), endoscopic grading and use of medication (Smith et al. 2014). Another study demonstrated that in CRSsNP and without asthma, need for oral corticosteroid therapy more than once in two years is more risky than ESS (Leung, Dinnie & Smith 2014). Operative management of CRS refractory to conservative therapy calls for the suspicion of asthma in CRSwNP patients (Hakansson et al. 2014)

In our study reported antibiotic courses for doctor-diagnosed were used as a sign of exacerbation-rate, during potoperative follow-up. The exacerbation-rate began to increase 9 months postoperatively, and this trend was observed throughout the follow-up period (in average 68-months). It is now known that full postoperative healing takes months and up to over a year following surgery (Verim et al. 2014). Also partly unknown immunological pathomechanisms of CRS may influence this, which cannot fully be controlled by performing sinus surgery. Patients may have a tendency to have recurrent acute sinusitis. It has been found that CRS patients with persisting symptoms may have impaired responses to protein vaccines, which might indicate that patients suffering from moderate to severe CRS have increased susceptibility to infections due to genetic or other unknown reasons (Tahkokallio et al. 2011, Wang et al. 2008). Similarly to this, locally impairment in immunity has been reported in eosinophilic upper airway diseases, including CRSwNP and AR (Hupin et al. 2013).

Our study indicates that patients with asthma or job exposure might experience less satisfaction with any procedure or might benefit more from maxillary sinus surgery with the ostium-enlarging technique, compared to patients without these risk-factors. This is in line with previous observations that risk factors affect the need for revision ESS, or subjective outcomes after balloon sinuplasty (Hox et al. 2012, Koskinen et al. 2012). Different bacterial colonization, biofilms, changes in innate immunity and/or other factors might play a role in CRS pathogenesis especially in patients with asthma and/or occupational exposure (Jardeleza et al. 2013, Schicht et al. 2013, Wang, Du & Zhao 2014). On the other hand, others have shown that allergic rhinitis without other risk factors would not seem to affect CRS severity or outcomes of ESS, as long as

atopy is taken into account and treated (Ryan 2008, Robinson, Douglas & Wormald 2006, Smith et al. 2005, Emanuel, Shah 2000). Furthermore, presence or absence of AR does not seem to affect endoscopically made diagnosis of CRS (Tomassen et al. 2011).

There are limitations to our studies. The anti-IDO mAb we used cannot discriminate between IDO1 and IDO2 isotopes, which might have different roles (Lob et al. 2009). Much of the existing literature does not yet discriminate between IDO1 and IDO2 (Munn, Mellor 2013). Moreover, we did not measure tissue IDO expression on micro RNA level, and the local activity of IDO by measuring the kyn/trp ratio in tissues. The clear weakness of studies IV-V is the small size of the study population. We also acknowledge that patients cannot side-specifically discriminate for all nasal symptoms such as postnasal drip and sense of smell. Also, follow-up period varied between patients and postoperative visits were performed at different times, and not necessarily by the same operating physician.

In the future, a great challenge would be to find new biomarkers and tools for prediction of disease susceptibility and its control. The initial discoveries regarding IDO made it an interesting and potential target in the investigation of inflammatory airway diseases. IDO might have a role in the nasal polyp pathomechanisms instead of asthma or pollen AR. Yet, more studies are required to bring evidence on this. CRS, similarly to asthma, follows a fluctuating course. When considering operative management of CRS, surgical decision must be based on appropriate findings and patient history. Furthermore, surgical procedure and postoperative care have to be performed according to current treatment guidelines. It seems that CRS patients, especially those with risk factors such as asthma and/or job exposure, could benefit more from an ostium enlarging approach than uncinectomy alone. Prospective controlled studies with large patient numbers are needed to observe which factors affect progression of CRS and long-term outcomes of ESS patients.

7. CONCLUSIONS

- Local expression of IDO associates to CRSwNP, but not to CRSsNP, or AR. Moreover a novel discovery was that CRSwNP is related to systemic level changes in immunity, detected by serum IDO activity. Whether local and systemic alterations in IDO pathways are a cause or consequence of CRSwNP, requires further studies.
- Altered IDO activity did not associate with asthma.
- Early endoscopic recovery following maxillary sinus surgery with the ostium-enlarging technique is superior to the ostium-preserving technique.
- Endoscopic score and radiologic Lund-Mackay score for the maxillary sinus correlate at nine months after maxillary sinus surgery. Identical long-term symptom relief was achieved by both sinus procedures.
- CRS patients with concomitant asthma and/or job exposure, might be slightly more satisfied with middle meatal antrostomy compared to ostium-preserving maxillary sinus procedure at about five years postoperatively.

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ORIGINAL CONTRIBUTION

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Indoleamine 2,3-dioxygenase expression is associated with chronic rhinosinusitis with nasal polyps and antrochoanal polyps*

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SUMMARY

Chronic rhinosinusitis without and with nasal polyps (CRSwNP and CRSsNP), and antrochoanal polyps are different phenotypes with different pathomechanisms. Indoleamine 2,3-dioxygenase (IDO) is an enzyme expressed in many cells involved in the catabolism of the essential amino acid tryptophan to kynurenine. IDO might have a role in allergic airway inflammation. The aim was to evaluate if IDO expression is associated with CRSsNP, CRSwNP, or ACP. One hundred fifty specimens from the nasal cavity and sinus mucosa were immunohistochemically stained with mAb anti-IDO. The expression of epithelial and leukocyte IDO was associated with CRSwNP and ACP. The presence of ASA intolerance, asthma, atopy, smoking and use of medication did not significantly change the results. The different expression of IDO could putatively indicate the differences in the pathomechanisms of CRSsNP, CRSwNP and ACP. Further studies on the role of IDO in upper airways pathologies are required.

Key words: aspirin intolerance, chronic rhinosinusitis, eosinophil, indoleamine 2,3 dioxygenase, nasal polyp

INTRODUCTION

Chronic rhinosinusitis (CRS) is a heterogeneous group of inflammatory diseases of the nose and paranasal sinuses lasting for at least 12 weeks without resolution [(1)]. It is a significant health problem with a prevalence of 10-16 % [(1)]. It is frequently associated with asthma but their inter-relationship is poorly understood [(1,2)]. Hence, the global research goal is to acquire knowledge on patient phenotypes, subtypes, and the factors amplifying mucosal inflammation in CRS [(3)]. CRS with nasal polyps (CRSwNP) and without (CRSsNP) are considered to be phenotypes of CRS with possibly different aetiologies and pathomechanisms, although they may also be interpreted as different degrees of inflammation [(3,4)]. Nasal polyps (NP) appear as oedematous masses originating from the middle meatus and affect between 1 and 4% of the general population [(1)]. Hyperplasic CRS has been an ill-defined group, representing putatively an overlap between CRS and NP [(1)]. Although CRS with hypertrophic polypoid sinus mucosa (HP) is also

Footnote: Honkanen & Luukkainen contributed equally to this manuscript

Abbreviations: ACP = antrochoanal polyp; ASA = acetylic salicylic acid, aspirin; CRS = chronic rhinosinusitis; CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; HP = hypertrophic polypoid sinus mucosa; IDO = Indoleamine 2,3-dioxygenase; Ig = immunoglobulin ; NP = nasal polyp; Th1 = T-helper cell 1; Th2 = T-helper cell 2

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lacking clinical characterization, its histological characters seem to be tortuous edematous mucosa with a putatively non increased number of glands, vessels, or eosinophils [5].

Sinus specimens obtained from patients suffering from CRSsNP are generally characterised by basement membrane thickening, goblet cell hyperplasia, subepithelial oedema, abundant mononuclear cells and few eosinophils [1,6]. Histomorphological characterisation of CRSwNP reveals frequent epithelial damage, a thickened basement membrane, oedematous to sometimes fibrotic stromal tissue, with a reduced number of vessels and glands, but virtually no neural structure [7]. Eosinophil numbers are significantly higher in polyp tissue compared to CRSsNP [4]. CRSsNP is characterized by a T-helper cell 1 (Th1) polarization with high levels of interferon-gamma (IFN-γ) and transforming growth factor beta (TGF-γ), while CRSwNP is characterised by a T-helper cell 2 (Th2) polarization with high interleukin 5 (IL-5) and immunoglobulin E (IgE) concentrations [8]. A deficit in T regulatory cell capacity in CRSwNP might lead to a strong increase in Th1 and Th2 effector cell signals [9]. Antrochoanal polyps (ACP) originate (unilaterally) from the maxillary sinus mucosa and protrude into the choana. Although macroscopically similar to the classic NP, ACP is considered to have a predominance of neutrophils [10].

In patients with aspirin (ASA) sensitivity, 36-96% have CRSwNP [11,12]. CRSwNP in ASA hypersensitive patients is characterised by involvement of all sinuses and nasal passages and thicker hypertrophic mucosa with abundant eosinophils [13]. Although the pathogenesis of chronic eosinophilic inflammation of the airway mucosa and nasal polyps in ASA-sensitive patients does not seem to be related to intake of aspirin or other nonsteroidal anti-inflammatory drugs, it has been speculated that the pathomechanism underlying CRSwNP in ASA sensitive patients may be different from that in ASA tolerant patients [1].

Indoleamine 2,3 dioxygenase (IDO) is an intracellular enzyme that initiates the first and rate-limiting step of tryptophan breakdown along the kynurenine pathway [14]. IDO is widely expressed in a variety of cell types including leukocytes and tumour cells [15]. Initially the role of IDO was thought to be mainly antimicrobial by reducing the availability of the essential amino acid tryptophan in the inflammatory environment [16]. In the past years, IDO has emerged as an important regulator of the immune system; however, it is not known whether local IDO activity is beneficial or detrimental to inflamed tissues. IDO is induced by IFN-γ and other inflammatory cytokines during inflammation or as a consequence of normal tissue function [17]. IDO suppresses T cell activity and promotes T cell tolerance to further antigenic challenges, by promoting the differentiation of naïve CD4 T cells into regulatory T cells, regulated putatively by dendritic cells [18-22]. IDO seems to serve as a negative feedback loop or is not essential for Th1 response, but it plays a distinct role in up-regulating Th2 dominant immune responses [15,23]. Moreover, IDO has also been shown to down-regulate Th2 responses [24]. The role of IDO in modulating allergic airway inflammation has recently been investigated [25-28].

As there may be pathophysiological differences between CRSsNP, CRSwNP, and ACP, our objective was to compare the sinonasal epithelial and mucosal expression of IDO in these subgroups.

MATERIALS AND METHODS

Subjects

This study was carried out at the Department of Otorhinolaryngology, Tampere University Hospital, Finland and has been approved by the Hospital's Ethical committee. All the subjects were Caucasian. Subject groups are shown in Tables 1 and 2. The inclusion criteria of patients were: diagnosis of CRSsNP, CRSwNP, or ACP based on EPOS criteria of symptoms, endoscopic and sinus computed tomography findings [1]. The exclusion criteria were cystic fibrosis, and diseases with a severe impact on general immunity. The exclusion criteria of control subjects were: sinonasal disease (except mild allergic rhinitis), or any other disease requiring constant medication. Diagnosis of atopy was based on skin prick test positivity. Diagnosis of asthma was based on clinical features and pulmonary function tests. Diagnosis of ASA intolerance was made on the basis of a history of wheezing or asthma attacks precipitated by non-steroidal anti-inflammatory drugs.

The first set of specimens was taken from the nasal cavity. Control nasal mucosa was obtained from the inferior turbinate under local anaesthesia from 19 healthy, non-atopic volunteers who did not use any medication. Polyp specimens were obtained from patients for diagnostic purposes or undergoing endoscopic polypectomy with or without sinus surgery, under local or general anaesthesia. The maxillary sinus specimens were taken from different individuals than the specimens of the first set. Control sinus mucosa was obtained from 12 subjects under general anaesthesia during bimaxillary osteotomy. Maxillary sinus specimens from patients with CRS were obtained during endoscopic sinus surgery under local or general anaesthesia.

Tissue handling

Nasal specimens, obtained from healthy volunteers, were immediately snap-frozen in liquid nitrogen and stored at -80°C until analysis. Other specimens, obtained at the time of surgery, were formalin-fixed and paraffin-embedded.

Sample staining

Tissue samples were stained with hemalaun-eosin for calculating the number of mucosal eosinophils/mm^2. The polyp specimens were additionally stained with Periodic acid-Schiff (PAS) for evaluating semiquantitatively the amount of mucus on the polyp surface (PAS+ = little or no mucus, PAS++ = much mucus).

Immunohistochemistry

For light microscopic immunoperoxidase staining, 3-5 µm thick frozen or paraffin sections were cut onto Superfrost Plus microscope slides (Menzel-Gläser, Braunschweig, Germany). Frozen sections were fixed with formalin for 45 minutes. Fully automated immunostaining was performed by Ventana BenchMark LT Automated IHC Stainer (Ventana Medical System, Arizona, USA). Ultraview Universal DAB detection kit (catalogue No. 760-500, Ventana Medical System, Arizona, USA) was used. Ventana EZ Prep solution (catalogue No 950-100, Ventana) was used for deparaffinisation. For epitope retrieval CC1: Tris-EDTA buffer pH 8.0 (catalogue No 950-124,Ventana) was used at 95°C to 100°C for 30 minutes with paraffin embedded tissue sections, and 8 minutes with frozen sections. Endogenous peroxidase was blocked with UV-Inhibitor 3% H_20_2 (Ventana) for 4 minutes at 37°C. Tissue slides were rinsed between steps with Ventana Tris-based Reaction buffer (catalogue No. 950-300, Ventana). Slides were incubated at 37°C for 32 minutes with mAb anti-Indoleamine 2,3 dioxygenase (1:200, clone MAB5412, Chemicon International Inc., USA) followed by application of Ventana Ultraview HRP Universal Multimer (8 minutes at 37°C). Diaminobenzidine (DAB) was used as a chromogen and haematoxylin as a nuclear stain. Known positive tissue samples

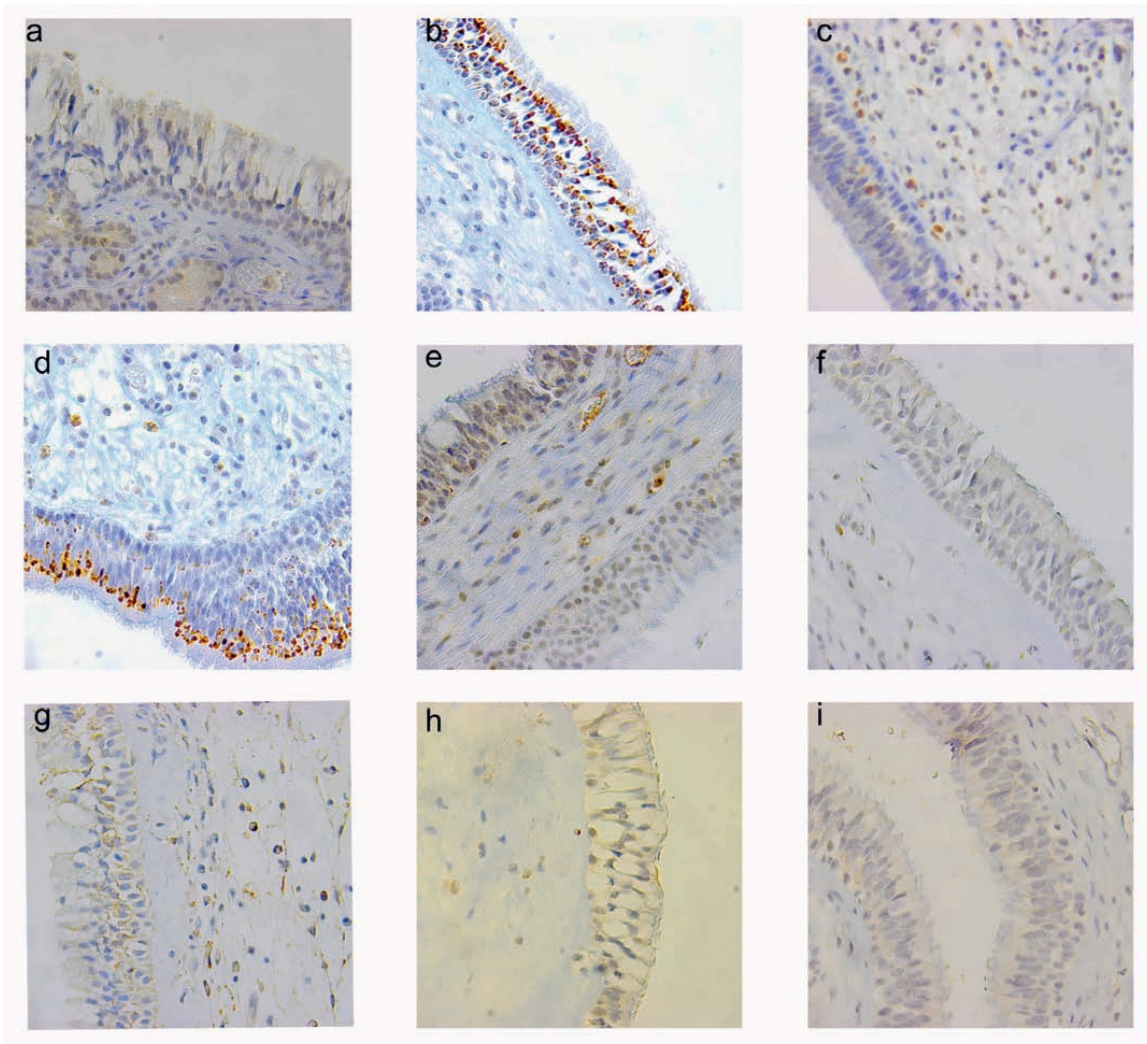

Figure 1. The expression of indoleamine 2,3-dioxygenase (IDO) in biopsies from the nasal cavity (a-d) and maxillary sinus mucosa (e-i). (a) Control nasal mucosa without epithelial or leukocyte expression of IDO. (b) Chronic rhinosinusitis with nasal polyp (CRSwNP) with epithelial IDO+ and leukocyte IDO-. (c) ASA-intolerant CRSwNP with epithelial IDO- and leukocyte IDO+. (d) Antrochoanal polyp with epithelial IDO+ and leukocyte IDO+. (e) Control sinus mucosa with epithelial IDO- and leukocyte IDO+. (f) CRSsNP with epithelial IDO- and leukocyte IDO-. (g) CRSsNP with epithelial IDO- and leukocyte IDO+. (h) CRSwNP with epithelial IDO- and leukocyte IDO+. (i) ASA-intolerant CRSwNP with epithelial IDO- and leukocyte IDO-. Original magnification 400x.

(from coeliac or inflammatory bowel disease) were also used to confirm the staining reliability of all separate staining patches [29,30]. The specificity of immunohistochemistry was controlled by omitting the primary antibodies or replacing them with irrelevant antisera.

Light microscopic evaluation

Sections were examined with a Leica DM 2000 light microscope (Leica Microsystems GmbH, Wetzlar, Germany) by two independent observers blinded to the experimental conditions. In a sample, there was either moderate or strong immunoreactivity of all epithelial cells or no epithelial positivity at all. Thus, the results were expressed as IDO positive epithelium or IDO negative epithelium. The percentage of IDO positive leukocytes was assessed. The number of eosinophils/mm^2 was counted from Hemalaun-Eosin staining. Diagnosis of hypertrophic polypoid mucosa (HP) of the maxillary sinus was based on microscopic evaluation of Hemalaun-Eosin stained sinus specimens by two blinded observers (T.P. and S.T-S). HP was semiquantitatively graded in specimens from patients with CRS: HP- = no or mild tortuous edematous mucosa, mild epithelial damage; CRS HP+ = moderate or severe tortuous edematous mucosa, moderate to severe epithelial damage.

Data analysis

Statistical analysis was carried out by the SPSS Base 15.0 Statistical Software Package (SPSS Inc., Chicago, IL, USA). Data is expressed as medians or means. For comparisons, the results were analysed by, Fisher's exact, Kruskal-Wallis, Mann Whitney U tests, and binary logistic regression analysis. Two-tailed p-values of < 0.05 were considered statistically significant.

RESULTS

Expression of IDO in the nasal cavity: control inferior turbinate, CRSwNP, and ACP

IDO was strongly expressed in the vicinity of the Golgi apparatus of epithelial cells, but not on the supraepithelial mucus (Figure 1). IDO was additionally expressed weakly in submucosal leukocytes and intraepithelial glands (Figure 1).

Compared to control inferior turbinate, epithelial IDO+ was significantly associated with CRSwNP and ACP ($p < 0.01$, by logistic regression analysis, Table 3). The findings remained the same, when adjusted by aspirin-intolerance, atopy, asthma, smoking, use of intranasal or peroral corticosteroids, or antihistamines, previous operations, recurrence of polyps, sex and age ($P < 0.05$, by logistic regression analysis, data not shown).

Compared to control inferior turbinate, the percentage of IDO positive leukocytes was significantly higher in CRSwNP and ACP specimens ($p < 0.001$, $p < 0.05$, respectively, by Kruskal-Wallis and Mann Whitney U test, Table 1). There were no differences in the median percentage of IDO positive leukocytes between the existence or not of the following factors: aspirin-intolerance, atopy, asthma, smoking, use of intranasal or peroral corticosteroids, or antihistamines, previous operations, recurrence of polyps, and sex ($p > 0.05$ by Kruskal-Wallis test, data not shown). Age did not correlate with the percentage of IDO positive leukocytes ($p > 0.05$ by Spearman rank correlation test, data not shown). The median percentage of IDO positive leukocytes was higher in specimens with epithelial IDO+ than that with epithelial IDO- (median percentages of IDO positive eosinophils 13% and 0%, respectively, $p < 0.001$ by Kruskal-Wallis and Mann Whitney U tests, data not shown).

The median number of mucosal eosinophils was higher in CRSwNP and ACP specimens compared to control inferior turbinate ($p < 0.001$ by Kruskal-Wallis and Mann Whitney U test, Table 1). The median number of eosinophils was significantly higher in epithelial IDO+ than in epithelial IDO- groups (median numbers of mucosal eosinophils 110.4/mm^2 and 0.04/mm^2, respectively, $p < 0.001$ by Kruskal-Wallis and Mann Whitney U test, data not shown). Moreover, the number of eosinophils/mm^2 correlated significantly with the percentage of IDO+ leukocytes ($p < 0.01$, $r = 0.41$, by Spearman rank correlation test, data not shown).

The epithelial IDO+ was associated with high amount of supraepithelial mucus, e.g. strong PAS positivity ($p < 0.05$, by Fisher's exact test, data not shown). A higher number of eosinophils was also significantly associated with the high amount of supraepithelial mucus (median numbers of mucosal eosinophils 171.2/mm2 and 65.6/mm2 in PAS++ and PAS+ groups respectively, $p < 0.05$ by Kruskal-Wallis and Mann Whitney U test, data not shown). In contrast, the median percentage of IDO positive leukocytes did not significantly associate with the amount of supraepithelial mucus ($p > 0.05$ by Kruskal-Wallis test, data not shown).

Expression of IDO in the maxillary sinus mucosa: control, CRSsNP, and CRSwNP

IDO was expressed in the sinus epithelium and submucosal leukocytes in a similar way as in the specimens taken from the nasal cavity (Figure 1).

Compared to control sinus mucosa, the epithelial IDO+ did not statistically differ in CRSsNP or CRSwNP groups ($p > 0.05$, by Fisher's exact test, Table 2, and by logistic regression test, data not shown). These findings remained insignificant when adjusted to aspirin intolerance, asthma, atopy, smoking, previous operations, microscopic evidence of hypertrophic polypoid sinus mucosa, use of intranasal or peroral corticosteroids, sex, and age ($p > 0.05$, by logistic regression analyses, data not shown).

In contrast, the median percentage of IDO positive leukocytes was significantly higher in CRSwNP specimens compared to both control sinus mucosa and CRSsNP groups ($p < 0.05$, by Kruskal-Wallis and Mann Whitney U test, Table 2). There were no differences in the percentage of IDO positive leukocytes between the existence or not of the following factors: aspirin-intolerance, atopy, asthma, smoking, use of

Table 1. Patient characteristics and results of specimens taken from the nasal cavity.

	Control n = 19	CRSwNP n = 54	ACP n = 10	p value
Age				
median	26	59	45	< 0.001*
min-max	21-62	16-90	20-79	
No. of male sex	4	36	7	0.002
Atopy	0	11	1	NS
ASA intolerance	0	13	0	0.012
Asthma	1	19	0	0.003
Smokers	1	11	1	NS
Medication				
Antihistamine	0	2	0	NS
Intranasal CCS ±antih.	0	28	3	< 0.001
Peroral CCS	0	5	0	NS
ESS performed	0	26 8	4	< 0.001
Recurrent NP	0		0	NS
No. of eosinophils/mm2				
median	0.0	152.0	108.0	< 0.001*
Q1-Q3	0.0-0.0	64.8-388.0	17.6-171.2	
Percentage of IDO+ leukocytes				
median	0.0	12.0	9.5	< 0.001*
Q1-Q3	0.0-1.5	8.0-25.0	4.0-17.0	
Specimens with				
epithelial IDO+	16 %	87 %	70 %	< 0.001
supraepithelial PAS++	0 %	62 %	56%	< 0.001

Control = mucosa from inferior turbinate from patient without inflammation of nasal mucosa and without sinonasal disease, CRSwNP = chronic rhinosinusitis with nasal polyp, ASA = aspirin, ACP = antrochoanal polyp, CCS = corticosteroid, antih. = antihistamine, ESS = endoscpic sinus surgery, Q1- Q3 = 25 and 75 percentiles, respectively, IDO+ = positivity with mAb anti-indoleamine 2,3 deoxygenase, PAS++ = strong Periodic acid shiff –staining positivity, indicating much supraepithelial mucus. P-values by Fisher´s exact test (discrete) or *Kruskal Wallis test (continuous). NS = not significant.

Table 2. Patient characteristics and results of specimens taken from the maxillary sinus.

	Control n = 12	CRSsNP n = 41	CRSwNP n = 14	p value
Age				
median	28	51	46	0.003*
min-max	19-56	16-75	16-76	
No. of male sex	4	13	9	NS
Atopy	5	25	8	NS
ASA intolerance	0	0	7	< 0.001
Asthma	0	13	8	0.003
Smokers	3	9	2	NS
Medication				
Antihistamine	0	4	2	0.067
Intranasal CCS± antih.	0	16	7	0.001
Peroral CCS	0	1	6	< 0.001
≥1 previous ESS	0	10	4	NS
Hypertrophic polypoid mucosa	0	11	14	< 0.001
No. of eosinophils/mm2				
median	80.0	82.7	72.6	NS *
Q1-Q3	0.0-0.0	0.0-0.0	0.0-16.0	
Percentage of IDO+ leukocytes				
median	0.0	0.0	5.0	< 0.001*
Q1-Q3	24.0-160.0	48.0-261.3	31.6-198.3	
Specimens with				
epithelial IDO+	42 %	51 %	71 %	NS

Control = maxillary sinus mucosa from patient without chronic rhinosinusitis, CRSsNP = chronic rhinosinusitis without nasal polyps, CRSwNP = chronic rhinosinusitis with nasal polyps, ASA = aspirin, CCS = corticosteroid, antih. =antihistamine, ESS = endoscopic sinus surgery, Q1- Q3 = 25 and 75 percentiles, respectively, IDO+ = positivity with mAb anti-indoleamine 2,3 deoxygenase. P-values by Fisher´s exact test (discrete) or *Kruskal Wallis test (continuous). NS = not significant.

Table 3. Comparison of epithelial IDO+ in specimens from nasal cavity.

	OR	OR CI		p-value
		lower	upper	
Control	ref			
CRSwNP	35.8	10.5	122.6	< 0.001
ACP	12.4	2.7	57.8	0.007

Non-adjusted odds ratio (OR), and lower and upper 90 % confidence intervals (CI) for comparison of epithelial IDO+ in specimens from nasal cavity.

intranasal or peroral corticosteroids, or antihistamines, previous operations, and sex ($p > 0.05$ by Kruskal-Wallis test, data not shown). Age did not correlate with the percentage of IDO positive leukocytes ($p > 0.05$ by Spearman rank correlation test). There was not significant difference in the median percentage of IDO positive leukocytes in epithelial IDO+ and epithelial IDO- groups ($p > 0.05$ by Kruskal-Wallis test, data not shown).

Compared to control sinus mucosa, the number of mucosal eosinophils did not differ in CRSsNP or CRSwNP groups ($p > 0.05$ by Kruskal-Wallis test, Table 2). The median number of eosinophils did not differ between epithelial IDO+ and epithelial IDO- groups ($p > 0.05$ by Kruskal-Wallis test, data not shown). The number of eosinophils /mm^2 did not correlate with the percentage of IDO positive leukocytes ($p > 0.05$, by Spearman rank correlation test, data not shown).

DISCUSSION

We demonstrated that in the nasal cavity, epithelial IDO+ and the percentage of IDO+ leukocytes were enhanced in CRSwNP and ACP groups, when comparing to control inferior turbinate. In contrast, when observing the maxillary sinus mucosa, epithelial IDO+ did not differ significantly in CRSsNP or CRSwNP groups compared to control sinus mucosa, whereas the percentage of IDO positive leukocytes was enhanced in CRSwNP group.

In the nasal cavity, enhanced epithelial IDO+ and a higher percentage of IDO+ leukocytes during CRSwNP and ACP, but not in control nasal mucosa, might have a role in the pathogenesis of nasal polyp subgroups. By reducing locally the essential amino acid tryptophan, IDO might suppress the activation of inflammatory cells in the epithelial wall and mucosa of polyp tissue thus having putatively a role in decreasing inflammation and tissue damage. Because we did not observe the specimens from inflamed turbinate mucosa from CRSwNP patients, it remains unknown whether IDO+ is characteristic of polyp tissue or other inflammatory processes also.

We found eosinophils most abundantly during CRSwNP than in controls or ACP, which is in line with the previous observations [1,4]. Previously, it has been shown that the gene encoding the IDO genotype found from nasal polyp specimens might have a role in the genetic risk for ASA intolerant asthma that was observed from serum samples of different patients [31]. Here, ASA intolerance did not significantly affect the results. We demonstrated that, epithelial IDO+ and the high median percentage of IDO positive leukocytes associated significantly to each other and to a higher median number of eosinophils, which could support that both epithelial and leukocyte IDO might have an immunological role in polyp tissue especially if there is a predominance of eosinophilic inflammation. Whether the role of IDO is to promote or inhibit inflammation is not known [32]. The role of IDO in certain leukocytes might be related to the promotion of T cell tolerance; and in eosinophils, the function of IDO is either stimulatory or inhibitory depending on target cell and stimulus [24,33,34]. Further studies are required to analyse functionally active IDO in activated leukocyte subtypes and related cytokines during CRSwNP and CRSsNP.

In contrast to the nasal cavity, high numbers of control subjects had epithelial IDO+ in the maxillary sinus. Patient history factors were not associated with the IDO+ in sinus controls. The role of IDO+ in control sinus specimens could include the suppression of bacterial growth or the promotion of tolerance. Yet, more studies are required to bring evidence on this.

Epithelial IDO+ was found in all specimens of the sinus mucosa, it was not associated to CRS subgroups. In contrast, a high percentage of IDO+ leukocytes was significantly associated with CRSwNP. A study with IDO-/- mice did not reveal a role for IDO in antigen-induced immune tolerance in the airways but instead, IDO seemed to promote antigen-driven Th2 responses via effects on lung dendritic cells [15]. This is in line with our finding that a high expression of IDO seems to be associated with CRSwNP group. In this study, all sinus specimens from patients with CRSwNP and about one quarter of specimens from CRSsNP group had microscopic signs of hypertrophic polypoid maxillary sinus mucosa (HP), the presence of which did not significantly affect the IDO expression. Thus, the putative role of histological HP on the pathogenesis of CRS requires further experiments.

The lower number of eosinophils in the sinus mucosa compared to NP, found in this study, is in line with previous observations [1]. In contrast, we did not find significant differences in the eosinophil numbers between different CRS subgroups, whereas others have shown CRSwNP to be associated with an elevated mucosal eosinophil count [3]. We demonstrated that epithelial IDO+ and the high median percentage of IDO+ leukocytes did not associate significantly to each other or to a higher median number of eosinophils in sinus mucosa. This controversy could possibly mean that IDO has at least partly a different immunological role in the sinus mucosa than in the nasal mucosa or polyp tissue. Still, further studies are warranted.

Others have shown that after in vivo aeroallergen exposure, serum IDO activity was increased in asymptomatic atopics compared with either symptomatic atopic or nonatopic indi-

viduals [35]. When exposing in vitro monocyte-derived dendritic cells with house dust mite *Dermatophagoides pteronyssinus* 1, functionally active IDO decreased in cells from patients with house dust mite-sensitive asthma compared to nonatopic asthmatics [27]. Here we demonstrated that additional diagnosis of atopy did not statistically significantly affect the local IDO expression in CRS subgroups and ACP. In asthmatic patients, the baseline IDO activity in sputum has been shown to be significantly lower than control levels, but normal baseline activity was induced by using inhaled corticosteroids [36]. In this study, we observed that patient history of asthma or intranasal or peroral corticosteroid medication did not statistically significantly affect IDO expression in upper airways pathologies.

In conclusion, this study showed that in nasal cavity, enhanced epithelial and leukocyte IDO are associated with both CRSwNP and ACP, but the association was strongest in CRSwNP. In maxillary sinus, a higher percentage of IDO positive leukocyte is associated with CRSwNP but not with CRSsNP. The pathophysiological mechanisms underlying these findings require further studies.

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Indoleamine 2,3-dioxygenase expression in patients with allergic rhinitis: a case-control study

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Abstract

Background: Indoleamine 2,3-dioxygenase (IDO) is a tryptophan catalyzing enzyme. It has been suggested that it has a role in lower airway allergic inflammations, but its role in allergic rhinitis has not been investigated.

Objective: Our aim was to evaluate the expression of IDO in the nasal mucosa of allergic rhinitis patients allergic to birch pollen during peak exposure to birch pollen allergen and compare it to non-atopic patients.

Methods: IDO expression was immunohistochemically evaluated from nasal specimens obtained in- and off-season from otherwise healthy non-smoking volunteers both allergic to birch pollen (having mild or moderate allergic rhinoconjunctivitis) and non-allergic controls.

Results: The IDO expression levels were low in healthy controls and remained low also in patients allergic to birch pollen. There were no differences in the expression of IDO in- and off-season in either healthy or allergic subjects.

Conclusions: There is a controversy in the role of IDO in upper and lower airways during allergic airway disease. It seems that IDO is associated to allergic inflammations of the lower airways, but does not have a local role in the nasal cavity at least in mild or moderate forms of allergic rhinitis.

Keywords: Indoleamine 2,3-dioxygenase, allergic rhinitis, birch pollen, dendritic cell, tryptophan, kynurenine, interferon gamma, leukocyte, eosinophil

Introduction

Indoleamine 2,3 dioxygenase (IDO) is an intracellular enzyme that initiates the first and rate-limiting step of tryptophan breakdown along the kynurenine pathway [1]. IDO is widely expressed in a variety of cell types including leukocytes and tumour cells [2]. Initially the role of IDO was thought to be mainly antimicrobial by reducing the availability of the essential amino acid tryptophan in the inflammatory environment [3]. In the past years, IDO has emerged as an important regulator of the immune system; however, it is not known whether local IDO activity is beneficial or detrimental to inflamed tissues. IDO is induced by interferon γ (IFN-γ) and other inflammatory cytokines during inflammation or as a consequence of normal tissue function [4]. IDO suppresses T cell activity and promotes T cell tolerance to further antigenic challenges, by promoting the differentiation of naïve CD4 T cells into regulatory T cells, putatively by regulation by dendritic cells [5-10]. IDO seems to serve as a negative feedback loop or is not essential for Th1 responses, but plays a distinct role in up-regulating Th2 dominant immune responses [2,11]. Moreover, IDO has also been shown to down-regulate Th2 responses [12]. The role of IDO in the modulation of allergic airway inflammation has recently been investigated [13-16].

Our objective was to observe IDO expression levels in the nasal mucosa of allergic rhinitis patients allergic to birch pollen in relation to exposure to birch pollen allergen and compare it to healthy controls.

Materials and methods

Subjects

This study is a case-control study. It was carried out at the Department of Otorhinolaryngology, Tampere University Hospital, Finland and has been approved by the Hospital's Ethical committee. Written informed consent was obtained from all patients. The subjects were Caucasian.

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Patients were either atopic with allergic rhinoconjunctivitis symptoms, or non-atopic. Moreover, the patients did not have other diseases such as asthma. The diagnosis of birch pollen-induced allergic rhinitis was based on a history of seasonal allergic rhinitis during spring, clinical examination, and skin prick test positivity. Characteristics of the subject groups are shown in table 1. Specimens were taken from the nasal cavity. Biopsies from the anterior edge of the inferior turbinate were obtained with Fokkens' forceps under local anaesthesia. Specimens were taken during both winter (off season) and during peak allergen exposure in spring (in season, natural allergen exposure). Patients were not allowed to use their medication (antihistamine and/or nasal corticosteroids) for a minimum of 5 days before nasal biopsies were taken.

Sample staining

Nasal specimens were immediately snap-frozen in liquid nitrogen and stored at -80°C until analysis. Tissue samples were stained with hemalaun-eosin to calculate the number of mucosal leukocytes and eosinophils/mm^2 and to evaluate the inflammation score (0 = no inflammation, 1 = mild, 2 = moderate, 3 = severe inflammation) For light microscope evaluation, immunoperoxidase staining was used and specimens were cut into 3-5 μm thick frozen sections on Superfrost Plus microscope slides. (Menzel-Gläser, Braunschweig, Germany). Frozen sections were fixed with formalin during 45 minutes. Fully automated immunostaining was performed by Ventana BenchMark LT Automated IHC Stainer (Ventana Medical System, Arizona, USA). Ultraview Universal DAB detection kit (catalogue No. 760-500, Ventana Medical System, Arizona, USA) was used. For epitope retrieval CC1: Tris -EDTA buffer pH 8.0 (catalogue No 950-124, Ventana) was used at 95°C to 100°C for 8 minutes. Endogenous peroxidase was blocked with UV-Inhibitor 3% H202 (Ventana) for 4 minutes at 37°C. Tissue slides were rinsed

Table 1 Patient characteristics

	Control n = 15	Atopy n = 12	P value
Age			
median	23	24.5	NS
min-max	21-36	21-34	
No. of male sex	4	5	NS
Peripheral blood eosinophils			
median	0.23	0.15	NS
Q1-Q3	0.085-0.295	0.12-0.20	
% of peripheral blood eosinophils			
median	3.0	2.5	NS
Q1-Q3	2.0-4.0	2.0-4.0	
S-IgE			
median	27.0	182.0	< .001
Q1-Q3	18.5-58.5	67.0-410.0	
IgE birch			
median	< .35	62.5	< .001
Q1-Q3	< .35- < .35	12.0-161.0	
IgE timothy grass			
median	< .35	4.75	< .001
Q1-Q3	< .35- < .35	1.6-.6.1	
No. subjects having SPT positivity to any basic allergen	0	12	< .001
birch	0	12	< .001
timothy grass	0	9	< .001
other pollen	0	12	< .001
animal dander	0	9	< .001
house dust mite	0	0	NS
other basic allergens	0	0	NS
Biopsies were taken during			
winter	11	8	NS
spring	7	11	.019
both	3	7	NS

between steps with Ventana Tris-based Reaction buffer (catalogue No. 950-300, Ventana). Slides were incubated at 37°C for 32 minutes with mAb anti-Indoleamine 2,3 dioxygenase (1:200, clone MAB5412, Chemicon International Inc., USA) followed by application of Ventana Ultraview HRP Universal Multimer (8 minutes at 37°C). Diaminobenzidine (DAB) was used as a chromogen and haematoxylin as a nuclear stain. Known positive tissue samples (from coeliac or inflammatory bowel disease) were also used to confirm the staining reliability of all separate staining patches [17,18]. The specificity of immunohistochemistry was controlled by omitting the primary antibodies or replacing them with irrelevant antisera.

Light microscopic evaluation

Sections were examined with a Leica DM 2000 light microscope (Leica Microsystems GmbH, Wetzlar, Germany) by two independent observers blinded to the experimental conditions. In a sample, there was either moderate or strong immunoreactivity of all epithelial cells or no epithelial positivity at all. Thus, the results were expressed as IDO positive epithelium or IDO negative epithelium. The percentage of IDO positive mucosal leukocytes was counted.

Data analysis

Statistical analysis was carried out by SPSS Base 15.0 Statistical Software Package (SPSS Inc., Chicago, IL, USA). Data is expressed as medians or means. For comparisons, the results were analysed by, Fisher's exact (discrete) or Kruskal Wallis and Mann Whitney U tests (continuous). For pair-wise comparisons McNemar's and Wilcoxon tests were used. Two-tailed P-values of < 0.05 were considered statistically significant.

Results

IDO was expressed in the vicinity of the Golgi apparatus of epithelial cells, but not on the supraepithelial mucus. IDO was additionally expressed weakly in submucosal leukocytes and in intraepithelial glands. When observing the nasal biopsies, the number of specimens having positive epithelial IDO staining was not associated with atopy during either winter or spring (P > 0.05 by Fisher's test, Table 1, Figure 1). The percentage of IDO positive leukocytes did not associate with atopy in specimens taken during either winter or spring (P > 0.05, by Kruskal-Wallis test, Table 2, Figure 1). Nor did it associate with the expression of IDO in the epithelium (P > 0.05, by Kruskal-Wallis test, data not shown). The subjects did not have changes in the epithelial IDO expression or in the percentage of IDO positive leukocytes when comparing specimens taken during winter and spring from the same individuals (P > 0.05, by Wilcoxon test, Table 2). The median number of mucosal eosinophils was significantly higher in atopic than in non-atopic subjects only during symptomatic spring (P = 0.044, by Kruskal-Wallis and Mann Whitney U test, table 2), whereas the percentage of eosinophils did not significantly differ between atopic and non-atopic subjects (P > 0.05, by Kruskal-Wallis test, table 2). Interestingly, during spring the number of mucosal leukocytes and the percentage of IDO positive leukocytes correlated significantly (P < 0.05, r = 0.46, by Spearman rank correlation test, data not shown).

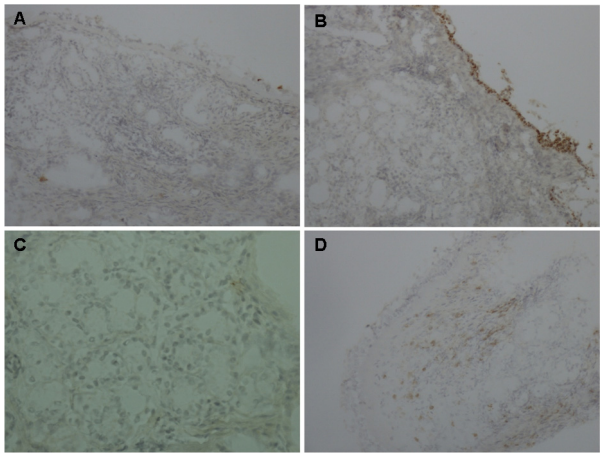

Figure 1 The expression of indoleamine 2,3-dioxygenase (IDO) in biopsies from the nasal cavity. (A) Nasal mucosa taken from a patient with allergic rhinitis off-season (during winter). No epithelial or leukocyte expression of IDO. (B) Specimen from another patient with allergic rhinitis, taken off-season, has epithelial IDO^+ and leukocyte IDO^-. (C) The same allergic rhinitis patient as in frame A, did not display any IDO expression in the specimen taken in-season (during spring). (D) Specimen from a healthy patient, taken off-season, shows epithelial IDO^- and leukocyte IDO^+. Original magnification 200× (A, B, D) and 400× (C).

As expected, atopics had significantly increased serum IgE levels (p < 0,001, by Kruskal-Wallis, Mann Whitney U). Atopic subjects had a significantly increased median symptom score during symptomatic spring in comparison to asymptomatic winter (P = 0.031, by Wilcoxon test, table 2), whereas in the non-atopic subjects, the median symptom score in winter and spring did not vary (P > 0.05, by Wilcoxon test, table 2). Accordingly, the median symptom score was significantly higher in the atopic than in the non-atopic group during spring but did not differ during winter (P = 0.003, P > 0.05, respectively, by Fisher's exact test, table 2).

Discussion

We aimed to evaluate IDO expression in two well defined human phenotypes: controls and their atopic counter-parts. Skin prick tests (SPT) were performed on

Table 2 Characteristics of nasal specimens

	Control winter n = 7	spring n = 11	P value[1]	Atopy winter n = 11	spring n = 8	P value[1]	P value[2]
Symptom score							
median	0	0	NS	0	2.0	.031	w NS*
Q1-Q3	0-0	0-0		0-0	1-2		s .003*
Inflammation score							
median	1.5	1.5	NS	1.0	1.5	NS	w NS*
Q1-Q3	1-2	1-2		1-2	1-3		s NS*
No. of leukocytes/mm^2							
median	640	1328	NS	1344	1328	NS	w NS
Q1-Q3	384-896	1280-1376		1120-1408	992-1760		s NS
No. of eosinophils/mm^2							
median	96	168	NS	144	248	NS	w NS
Q1-Q3	48-144	112-224		128-160	192-448		s .044
Percentage of eosinophils							
median	14.3	12.5	NS	12.0	25.5	NS	w NS
Q1-Q3	12.5-16.1	8.8-16.3		9.1-12.5	12.9-29.0		s NS
Percentage of IDO^+ eosinophils							
median	0.0	0.0	NS	0.0	0.0	NS	w NS
Q1-Q3	0.0-0.0	0.0-0.0		0.0-2.5	0.0-5.5		s NS
No. specimens with epithelial IDO^+	2	2	NS	1	2	NS	w NS* s NS*

Patient characteristics and results of specimens taken from the nasal cavity. Control = mucosa from inferior turbinate from patient without inflammation of nasal mucosa and without sinonasal disease, P value[1] = by pair-wise comparison between winter and spring by Wilcoxon test; P value[2] = comparison between control and atopy groups either in winter (w) or in spring (s) by *Fisher's exact test (discrete) or Kruskal Wallis test (continuous).

all study subjects to confirm diagnosis. The control and atopic groups did not differ in terms of patient number, age, sex, peripheral blood eosinophils, percentage of peripheral blood eosinophils. (p > 0,05 Kruskal-Wallis, Mann Whitney U) In comparison to controls, atopics had significantly higher serum IgE levels as well as significantly higher IgE levels against allergens to which the subjects were allergic. Atopics and controls differed in their symptom score only during the spring (in season, natural allergen exposure). Surprisingly, there were no differences between inflammation score, leukocyte numbers, percentages of eosinophils, IDO+ in epithelial cells and leucocytes during winter (off season) and spring. Atopics had a higher number of eosinophils during spring only.

We observed that in the nasal cavity, expression of IDO was low and did not differ between atopic and non-atopic groups. Moreover IDO expression did not fluctuate in the same individuals between symptomatic spring and asymptomatic winter season. We have also previously shown that the role of the nasal epithelium in birch pollen atopy is important [19]. In contrast others have shown that IDO might have a role in atopy. Paveglio et al. very recently showed in a transgenic mouse model that over-expression of IDO in the lungs might cause an anti-asthmatic effect by diminishing proliferation, numbers and cytokine production of CD4 + T cells [20]. Others have also previously shown that after *in vivo* aeroallergen exposure, serum IDO activity was increased in asymptomatic atopics compared with either symptomatic atopic or non-atopic individuals [21]. When exposing *in vitro* monocyte-derived dendritic cells with house dust mite Dermatophagoides pteronyssinus 1, functionally active IDO decreased in cells from patients with house dust mite-sensitive asthma compared to non-atopic asthmatics [15]. In asthmatic patients, the baseline IDO activity in sputum has been shown to be significantly lower than control levels, but normal baseline activity in asthmatic patients could be induced by using inhaled corticosteroids [22]. We have previously demonstrated that a high expression of epithelial and leukocyte IDO is associated with chronic rhinosinusitis with nasal polyps, while the expression in chronic rhinosinusitis without nasal polyps remained at the control level [23]. Thus in the upper airways, in contrast to lower airways, it seems that IDO might have a role in the nasal polyp pathomechanisms instead of atopy. Yet, more studies are required to bring evidence on this.

The expression of IDO differs between tissues [24]. Recently, two isoforms of IDO have been studied, IDO1

and IDO2. The expression and activity of both isoform differs in different tissues and in same cell cultures [25]. Both isoforms therefore might differ somehow in their roles [26,27]. Having this in mind, it could be possible that the anti-IDO mAb we used could be specific only for the IDO1 isotope [27]. Also, in some tissues IDO expression does not correlate with its activity. Indeed, elevated expression of IDO can translate into little activity or no activity at all [24]. Even though high levels of IDO expression can be measured, this does not necessarily translate into activity measured by ratio of tryptophan over kynurenine.

Both atopic asthma and atopic rhinitis are complex diseases with different phenotypes and genetic backgrounds. Currently it is thought that patients with certain (epi)genetic elements are prone to have immunological dysfunctions of respiratory barriers putatively by stimulation by certain environmental interactions [28]. Thus, our findings suggest that adult patients with only birch pollen AR but not asthma symptoms do not have local IDO. In the future, it would be of interest to observe whether immunological factors differ in patients having only the AR phenotype compared to those with the AR and asthma phenotype [29].

Conclusions

This study showed that in the nasal cavity, epithelial and leukocyte IDO expression is low and is not associated to allergic rhinitis caused by birch pollen. There is a controversy in the role of IDO in the pathogenesis of atopy, between upper and lower airways. More human studies are warranted to study the role of IDO in more detail in allergic rhinitis and other atopies.

Abbreviations

AR: allergic rhinitis; IDO: Indoleamine 2,3-dioxygenase; Ig: immunoglobulin; Th1: T-helper cell 1; Th2: T-helper cell 2.

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Authors' contributions

ST-S designed the study and collected the specimens with ML; TH performed staining. AL, ST-S, TP, and JK evaluated the specimens. AL and ST-S were involved in the data interpretation, literature search and writing. All authors commented critically on the writing. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Relationships of indoleamine 2,3-dioxygenase activity and cofactors with asthma and nasal polyps

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ABSTRACT

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Background: Asthma and chronic rhinosinusitis with nasal polyps (CRSwNPs) are coexisting diseases that are multifactorial. The rural environment seems to protect from atopy, but its relation with nonatopic airway inflammations has been less investigated. Indoleamine 2,3-dioxygenase (IDO) is an enzyme involved in the catabolism of the essential amino acid tryptophan (Trp) to kynurenine (Kyn). Low IDO activity has been previously observed in atopy and asthma. The objective was to investigate the relationships of IDO activity, eosinophils, and cofactors during asthma and/or CRSwNPs.

Methods: A Finnish population-based cohort of adult asthmatic patients (n = 245) and nonasthmatic patients (n = 405) was used. The presence of asthma and atopy were based on patient history and standardized diagnostic tests. The presence of acetyl salicylic acid intolerance, doctor-diagnosed NPs, and countryside environment during childhood were based on a questionnaire report. Serum IDO activity was evaluated by assessing the Kyn/Trp ratio by liquid chromatography.

Results: Low IDO activity was associated significantly with atopy, CRSwNPs, and an urban background. IDO activity did not correlate with pulmonary function. As expected, CRSwNPs was more frequent among asthmatic patients. A rural background has a protective effect from atopy and atopic asthma but it did not affect the prevalence of CRSwNPs or nonatopic asthma.

Conclusion: Low IDO activity might result from the urban environment and influence the development of the atopic phenotype. On the other hand, low IDO activity, found in CRSwNPs, does not seem to be related to the urban background and thus may result from other, still unknown, factors.

Keywords
Acetyl salicylic acid intolerance, asthma, atopy, indoleamine 2,3 dioxygenase, kynurenine, nasal polyp, tryptophan

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Asthma and chronic rhinosinusitis (CRS) are versatile, multifactorial inflammatory diseases of the airways that often coexist.[1–5] The prevalence of CRS in Europe is similar to that of asthma (~10%)[3,6–9] Asthma and/or CRS seem at least partly related to atopy, smoking, environmental exposure, imbalanced microbiome–barrier interaction, immunodeficiency, cilia dysfunction, and other endogenous factors.[10] The countryside environment protects from childhood asthma and atopy.[11] Moreover, there is a correlation between the prevalence of atopy and diversity loss of neighboring plants as well as that of the skin microbiota.[12]The relationship between countryside environment and CRS with nasal polyps (CRSwNPs) is not known. Previous studies have confirmed the importance of immune detection and Th2 cell–mediated immune responses in the pathogenesis of asthma and CRSwNPs.[3,13,14] On the other hand, CRS without nasal polyps (CRSsNP) is characterized by a T-helper 1 cell (Th1) polarization with high levels of interferon γ and transforming growth factor β.[15] Approximately 8% of asthmatic patients suffer from aspirin (ASA) hypersensitivity.[16] ASA-intolerant patients having asthma and/or CRS(wNPs) usually have a persistent and treatment-resistant form of disease, the pathogenesis of which might differ from that of ASA-tolerant patients.[3] ASA-intolerant CRS(wNPs) is characterized by involvement of all sinuses and nasal passages with hypertrophy and increased thickness of the mucosa with abundant eosinophilia (Eos).[17] Patients with ASA intolerance or other treatment resistant forms of airway inflammation might experience considerable morbidity and organ-threatening complications. Suffering and the substantial costs of these lifelong diseases may be reduced by finding biomarkers and tools for preventing progression of airways inflammation.

Indoleamine 2,3 dioxygenase (IDO) is an intracellular enzyme that initiates the first and rate-limiting step of tryptophan (Trp) breakdown along the kynurenine (Kyn) pathway. IDO is widely expressed in a variety of cell types, including leukocytes, antigen-presenting cells, and tumor cells.[18–20] In noninflammatory states, IDO seems to mediate tolerance to self.[21,22] IDO is induced in dendritic cells (DCs), which restrict infection and prevent exaggerated host responses.[23,24] Low IDO activity has previously been observed in atopy and asthma.[25,26] We previously showed in the sinonasal tract that epithelial and leukocyte expression of IDO is associated with CRSwNPs but not with allergic rhinitis.[27,28] The aim of this controlled study was to investigate the relationships of IDO activity, Eos, and cofactors during asthma and/or CRSwNPs.

MATERIALS AND METHODS

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Subjects

This study was perforemd at the Department of Otorhinolaryngology, Tampere University Hospital, Finland from 2001 to 2008. The subjects were Caucasian, without diagnosis of cystic fibrosis, and without other diagnoses or medication having a severe impact on general immunity. Characteristics of the subject groups are shown in Tables 1 and 2. Asthmatic patients and control subjects were participants in a Finnish population-based case–control study aimed at identifying risk factors and predictors of the outcome of adult asthma. Inclusion criteria for asthmatic subjects were age of >30 years and entitlement to special reimbursement for asthma medication from the Social Insurance Institution of Finland. The entitlement is granted if the criteria for persistent asthma are fulfilled, as certified by a chest specialist. Typical history, clinical features, and asthma course must be documented. At least one of the following physiological criteria is required for diagnosis: (1) a variation of 20% or greater in diurnal peak expiratory flow (PEF)

recording (reference to maximal value), (2) an increase of 15% or greater in PEF or forced expiratory volume in 1 second (FEV_1) with β_2-agonist, or (3) a decrease of 15% or greater in PEF or FEV_1 in exercise testing. Moreover, at least a 6-month period of continuous regular use of antiasthmatic medication must have elapsed by the time of the decision. One to two control subjects without asthma or chronic obstructive pulmonary disease were initially selected for each subject through a register covering the entire population. No other exclusion criteria were used for control subjects. Patients and control subjects were matched for age, sex, and area of residence. The basic characteristics of the patient and control groups are presented in Table 1.

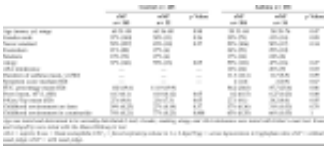

Table 1
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Table 1

Characteristics of the study population (*n* = 643)

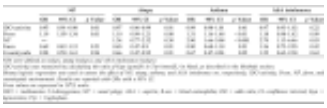

Table 2
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Table 2

The associations between serum IDO activity and NP, atopy, asthma and ASA intolerance as well as the number of peripheral B-eos, NP, the farm and the countryside environments

Approval for this study was obtained from the Ethical Committee at Tampere University Hospital. Written informed consent was obtained from all subjects.

Atopy was determined by means of skin-prick testing performed by specially trained nurses with a panel of 22 common allergen extracts (ALK A/S, Copenhagen, Denmark). These allergens were selected to cover exposures in both urban and rural environments. Skin-prick test responses were considered positive if at least one allergen caused a wheal with a diameter at least 3 mm larger than that produced by the negative control. Allergy testing by using the skin-prick test method was performed on 99.1% of asthmatic patients (93 male and 150 female patients) and 99.3% of control subjects (150 male and 252 female subjects). The environmental background was evaluated by the following questions: How often were you involved with animals in your childhood? Did you spend your childhood on a farm? Where did you live as a child (urban or countryside environment)? Both asthmatic and nonasthmatic subjects were asked if they had other allergic symptoms in addition to skin symptoms: allergic rhinitis, conjunctivitis, respiratory symptoms, other symptoms? ASA intolerance diagnosis was based on the questionnaire. Only asthmatic patients were asked “Have you ever had wheezing episodes after ingestion of ASA or another nonsteroidal anti-inflammatory drug (nonsteroidal anti-inflammatory drug [NSAID]), if yes, please specify.” For statistical analysis, patients were classified as having ASA intolerance if ASA or other NSAIDS-caused wheezing. The presence of NPs was asked from all patients by the question “Have nasal polyps ever been found in your nose?”[29] The symptom score of asthma was based on the patients' own subjective assessment on the severity of their asthma: 1 = mild to no symptoms, 2 = moderate, and 3 = severe symptoms.

Blood and Serum Samples

Kyn and Trp concentrations in the serum samples were measured by high-performance liquid chromatography. Trp was monitored by fluorescence detection at 266 nm excitation wavelength and 366 nm emission wavelength; Kyn was measured by ultraviolet absorption at 360 wavelength. Kyn/Trp ratio was calculated by relating concentrations of Kyn

(μmol/L) to Trp (mmol/L) allowing an estimate of IDO activity.[29,30]

The Eos count was performed using Technicon H3 analyzers (Bayer Diagnostica, Tarrytown, NY). Eos numbers are expressed as units in 10^9/L.

Statistical Analysis

Statistics were performed with SPSS Base 16.0 Statistical Software Package (SPSS, Chicago, IL). Data are expressed as means and as medians when specified. For comparisons, the results were analyzed by *t*-test, Mann-Whitney *U* test, Fisher's exact test, and binary logistic regression analyses. Results of comparisons by binary logistic regression are reported as odds ratios (ORs) with 95% confidence intervals (CIs).

Two-tailed values of $p < 0.05$ were deemed as being statistically significant.

RESULTS

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IDO Activity

Six hundred forty-three patients were enrolled in this study. Patient characteristics are shown in Table 1. Serum IDO activity did not associate with asthma or reported ASA intolerance (OR = 0.993; 95%CI = 0.98–1.01; $p = 0.406$; respectively, Tables 2 and 3) and IDO activity did not correlate with the FEV_1 percentage value, duration, and symptom score of asthma ($p > 0.05$; Spearman rank correlation test, data not shown).

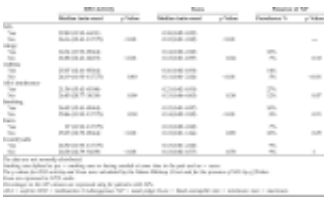

Table 3

The impact of measured factors on IDO activity, peripheral B-eos, and NPs

Table 3
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Low serum IDO activity was associated with patient-reported doctor-diagnosed CRSwNPs (OR = 0.946; 95% CI = 0.91–0.98; $p = 0.006$; Tables 2 and 3). The result remained the same, when adjusted to asthma, smoking, atopy, and ASA intolerance. On the other hand, low IDO activity was associated with atopy (OR = 0.973; 95% CI = 0.96–0.99; $p = 0.005$; Tables 2 and 3).[26] Also, this result remained the same when adjusted by asthma, smoking, ASA intolerance, and CRSwNPs.

Blood Eosinophilia

Increased blood eosinophilia (B-eos) were associated with asthma (Tables 2 and 3). The association was found only if the patient was nonatopic or had never smoked (data not shown). In addition, elevated B-eos were observed during patient-reported doctor-diagnosed CRSwNPs (Tables 2 and 3). The association was found only if the patient was nonatopic or had concomitant asthma or had never smoked (data not shown). B-eos was not associated with atopy or ASA intolerance (Tables 2 and 3). This result remained the same when adjusted for NPs, atopy, ASA intolerance, and smoking (data not shown).

Relationships between Asthma, CRSwNPs, and ASA Intolerance

The 14.3% of asthma patients and 5.4% of controls reported having doctor-diagnosed NPs, which differed significantly ($p < 0.001$, by Fisher's exact test; Tables 2 and 3). Atopy or smoking was not associated with NPs (Tables 2 and 3).

Only asthmatic patients were asked if they had worsening of asthma or increased wheezing when using NSAIDs. Thirty-three (13.9%) of 238 asthma patients had patient-reported ASA intolerance. Nine (27%) ASA-intolerant asthmatic patients had concomitant patient-reported doctor-diagnosed NsP. Thus, the prevalence of NP in ASA-sensitive asthma was 27%, and in ASA-tolerant asthma it was 12% ($p = 0.031$, by Fisher's exact test). Similarly, asthma patients with NPs reported more frequently coexisting ASA intolerance than asthma patients without NPs ($p = 0.031$, by Fisher's exact test; Table 1).

Effect of Rural Environment in Childhood

Both childhood spent on the farm or in the countryside protected subjects from atopy (Table 2). Farm environment during childhood was not associated with asthma, but countryside environment had a protective effect (Table 2). The protective effect was not observed, if the patients were not atopic (OR = 1.00; 95% CI, 0.59–1.70; $p = 0.99$). Childhood spent on the farm or in the countryside did not associate with patient-reported doctor-diagnosed CRSwNPs (Table 2). The result remained the same when adjusted by atopy or asthma (data not shown).

DISCUSSION

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In this study we showed that serum IDO activity was significantly lower in patients with NPs, independently of asthma, atopy, ASA intolerance, and smoking. We also showed lower IDO activity in atopic subjects in comparison with nonatopic subjects. This is in line with the findings of Raitala *et al.* who observed the nonasthmatic subpopulation of this cohort and showed also lower IDO activity in atopic aubjects.[26] On the other hand, we have previously shown local IDO expression associates with CRSwNPs but not with the allergic rhinitis phenotype.[27,28] There has previously been little evidence on the fact that NPs might affect immunity on a system level independently of its comorbidities.[31,32] Previous studies have shown a high serum IDO activity to be associated with immunosuppression in pathogenic tumorigenesis, renal insufficiency, pulmonary tuberculosis, celiac disease, and community-acquired pneumonia.[33–37] In NPs the indoleamine 2,3-dioxygenase genotype might have a role in the genetic risk for ASA-intolerant asthma.[38] Interestingly, we previously showed in a different population a high epithelial IDO expression in NP tissue both in patients with or without ASA intolerance.[27] The fact that patients with CRSwNPs might have a high level of epithelial IDO locally, but low serum activity of IDO, requires further elucidation. Local and systemically acting IDO probably have different immunoregulatory functions in patients with CRSwNPs. Two isoforms of IDO, IDO1 and IDO2, have recently been identified. Both differ in local expression, activity, and organ specificity.[39]

The function of IDO in Eos is either stimulatory or inhibitory on Th1 and Th2 cells depending on the inflammatory model and previous sensitization.[40,41] DCs can present antigens in an immunogenic or tolerogenic fashion, depending on environmental factors.[42] IDO has signaling activity in DCs, which are stably turned into regulatory DCs.[43] Others have shown that IDO in lung DCs promotes Th2-mediated allergic airway inflammation, but does not seem to be essential for immune tolerance *via* inhibition of Th1 response.[44,45] In a murine asthma model, immature DCs expressing IDO relieved allergic airway inflammation, as measured by decreased Eos and total cell counts, as well as by improved pulmonary histopathology.[46,47] Paveglio *et al.* showed in a transgenic mouse model that

overexpression of IDO in the lungs might cause an antiasthmatic effect by diminishing proliferation, numbers, and cytokine production of $CD4^+$ T cells.[48] Maneechotesuwan *et al.* showed that asthmatic patients have low baseline IDO activity in sputum and that IDO activity could be enhanced by treatment with inhaled corticosteroids.[25] Moreover, after *in vivo* aeroallergen exposure in asthma patients, serum IDO activity increases in asymptomatic atopics compared with either symptomatic atopic or nonatopic individuals.[49] In contrast, in our study we were not able to show that serum IDO activity is associated with asthma, its severity, or reported ASA intolerance.

NPs of Caucasian patients are characterized by pronounced local Eos[50] and systemically by increased B-eos.[51] Our findings of elevated B-eos was observed during patient-reported doctor-diagnosed CRSwNPs but only in those with concomitant asthma, which reflects that Eos might be mostly caused by asthma or both diseases rather than CRSwNP solely. This is in line with the findings that NPs in the presence of elevated serum Eos and sneezing act as predictors of bronchial hyperresponsiveness.[31,32] Keseroglu *et al.* found that serum IL-16, a potent inducer of Eos, was elevated in patients with NPs in comparison with controls. Hu also found in a Chinese cohort with either eosinophilic or noneosinophilic CRSwNPs that eosinophilic CRSwNP patients had elevated peripheral Eos absolute counts as well as blood IgE levels. Also, these patients tended to be of the male gender and have a higher prevalence of atopy and smoking.[52] Interestingly, we found an association between increased B-eos and an asthma diagnosis, although airway Eos is often heterogeneous among asthmatic patients.[53] Schwartz *et al.* showed that total Eos counts in peripheral blood reflects asthma activity and is useful for early detection of exacerbations.[54]

Others have shown that the prevalence of nasal polyposis in ASA-sensitive asthmatic patients may be as high as 60–70%, when compared with <10% in the population of ASA-tolerant asthmatic patients.[3] Interestingly, in our study population, the prevalence of NPs in ASA-sensitive asthma was only 27%.

We showed that, both farm and countryside environment during childhood protect from atopy, which is in line with previous observations.[11,12] Interestingly, the countryside environment but not a farm background protects from atopic asthma. On the other hand, the countryside environment did not seem to protect from nonatopic asthma and/or CRSwNPs. Kauffman *et al.* report that a lower prevalence of asthma is observable in individuals who have lived in the countryside before they were 16 years old in comparison with people who have not.[55] However, the authors do not specify the age of onset of asthma. Farming might even lead to nasal congestion, NPs, and nonatopic asthma.[56,57] Also, this protective effect was not found if the patient had nonatopic asthma and/or CRSwNPs. In addition, childhood on the farm or in the countryside did not have an effect on the prevalence of CRSwNPs. Thus, childhood spent on a farm or in the countryside may not provide protection from nonatopic inflammatory diseases of the respiratory tract and, specifically, the upper respiratory tract, Also, these backgrounds may provide protection up to a certain age, while the immune system matures; after a certain age moving from an urban environment to either a rural or a farm environment may not provide protection anymore.

The weakness of this study is that compared with the diagnostic methods of asthma and atopy, the diagnosis of NPs lacked nasoendoscopy. Another shortcoming is that the total time of exposure of the farm or countryside environment was not assessed, which would have provided more concrete information on their role.

CONCLUSION

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In the future, a great challenge would be to find new biomarkers and tools against inception and loss of control during asthma and/or CRSwNPs. Low IDO activity might result from urban environment and influence the development of the atopic phenotype. On the other hand, low IDO activity, found in CRSwNPs, does not seem to be related to an urban

background and thus may result from other, still unknown, factors. These findings seem to be independent of asthma or ASA intolerance. The role of IDO in the pathophysiology of NPs remains open. The future directions for this study would be to evaluate the clinical possibilities of these findings.

NOTES

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Research Article

Endoscopic Sinus Surgery with Antrostomy Has Better Early Endoscopic Recovery in Comparison to the Ostium-Preserving Technique

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Background. Endoscopic sinus surgery (ESS) is considered for chronic rhinosinusitis (CRS) after failure of conservative therapy. *Objective*. The aim of this study was to evaluate endoscopically ostium patency and mucosal recovery after ESS, with either maxillary sinus ostium-preserving or -enlarging techniques. *Materials and Methods*. Thirty patients with non-polypous CRS were enrolled. Uncinectomy-only and additional middle meatal antrostomy were randomly and single-blindly performed for each side. Pre- and postoperative endoscopic scores were semi-quantitatively determined according to findings in the ostiomeatal complex area. Adhesions, maxillary sinus mucosal swelling, secretions, and ostium obstruction were also endoscopically evaluated. In addition, symptoms were asked and computed tomography scans were taken preoperatively and 9 months postoperatively. *Results*. At 16 days postoperatively, a better endoscopic score and a less obstructed ostium were found with antrosomy. At 9 months postoperatively the endoscopic score improved significantly and identically with both procedures, however, obstructed ostia and sinus mucosal swelling/secretions were insignificantly more frequently found on the uncinectomy-only side. Endoscopic and radiologic findings of the maxillary sinus mucosa and ostium correlated significantly 9 months postoperatively. *Conclusion*. There was a good long-term mucosal recovery with both surgical procedures. In terms of early mucosal recovery and ostium patency, antrostomy might be slighly superior.

1. Introduction

Chronic rhinosinusitis (CRS) is an inflammation of the nose and paranasal sinuses lasting more than 12 weeks with a prevalence of about 10% in Europe [1, 2]. It is diagnosed by typical symptoms and/or computed tomography (CT) scan and/or endoscopic changes [1]. After failure of conservative therapy, endoscopic sinus surgery (ESS) aims to restore mucociliary clearance and ventilation through the natural ostia. ESS is based on the theory that the maxillary sinus ostium is the most important area in the pathogenesis of chronic and recurrent rhinosinusitis [3, 4]. Obstruction of the ostium is believed to lead to chronic inflammation and eventually to pathologic alterations of the maxillary sinus mucosa. Therefore, surgical opening of the ostium and thus improved drainage and ventilation of the sinus might restore the normal mucosa [5]. There are different opinions concerning the extent of surgery of the ostiomeatal complex. It is considered that removal of the uncinate process alone would be enough to restore the ventilation of the maxillary

sinus. ESS with the minimally invasive technique aims to achieve normal sinus function and prevent sinus exposure to environmental irritants, by causing minimal opening of the sinonasal structures [6, 7]. The effect of minimally invasive ESS has been shown to be comparable to invasive ESS [6, 8–10]. Only few controlled studies have compared small or no widening of bony or cartilaginous structures in the maxillary sinus ostium to antrostomy with relatively promising results [11–15]. On the other hand, uncontrolled studies suggest that the presence of biofilms, osteomyelitis, and other factors favor invasive approaches towards the ostiomeatal unit [16, 17].

Our aim was to compare endoscopically the mucosal recovery of the ostiomeatal complex area and maxillary sinus, after endoscopic sinus surgery with either the ostium—preserving or ostium—enlarging technique.

2. Materials and Methods

2.1. Subjects. This randomized, single-blinded study was carried out in the Department of Otorhinolaryngology, Tampere University Hospital, Finland, and Mikkeli Central Hospital, Mikkeli, Finland, between 2001 and 2003.

Characteristics of groups of patients can be seen in Table 1. Thirty patients with CRS were enrolled in this study.

Inclusion criteria were moderate-to-severe sinus-related symptoms, according to patient interview,during at least 12 weeks despite maximal medical treatment and a Lund-Mackay (LM) sinus computed tomography (CT) score [18] of at least 6/24 but no more than 18/24.

Exclusion criteria were age less than 18 years; oral corticosteroid treatment during the last two months prior to surgery; previous sinonasal surgery; a history or physical examination suggestive of severe nasal septal deviation (that causes only unilateral nasal obstruction and/or requires septoplasty before ESS can be performed), unilateral sinusitis, nasal polyposis > grade 1 [18], aspirin sensitivity, chronic bronchitis, cystic fibrosis, a tumour or a disease with a severe impact on general immunity.

Dropouts from the study one patient died accidentally prior to the control at 9 months postoperatively.

2.2. Sinus Surgery. ESS was performed by two authors Myller et al. as previously described [13, 15, 19]. Briefly, the uncinectomy was performed on both sides, in which the lower two-thirds of the uncinate process was removed. Additional middle meatal antrostomy was randomized on either the right or the left side of each patient. It was performed by removing with cutting forceps, the posterior connective tissue of the natural ostium, to duplicate the diameter. If a large ethmoid bulla was disturbed doing uncinectomy and/or antrostomy, it was opened. The light middle meatal tamponation was removed on the first postoperative day. Nasal endoscopy was performed, and the operation field was cleaned 2 weeks after surgery (Table 1).

2.3. Endoscopy. The endoscopic evaluation was performed-preoperatively, 1–77 days (mean ± SD, 26 ± 23 days) before the operation, perioperatively, and during the debridement follow-up visit at 7–30 days (mean ± SD, 16 ± 5 days) postoperatively, 3 and 9 months postoperatively. Physicians filled a form with endoscopic findings. The maxillary sinus ostium obstruction was scored: 0: no and 1: yes. The maxillary mucosa was scored: 0: normal (with or without cyst), 1: edema, and 2: polypous mucosa. The maxillary mucosal sinussecretions were scored: 0: no, 1: mucus, and 2: pus. The endoscopicscore of the ostiomeatal complex area was semiquantitatively determined from the following changes: swollen/polypotic mucosa found in the middle turbinate/anterior ethmoid cells/uncinate process/maxillary sinus ostium/opening of the frontal recess; middle meatal adhesions; anatomical narrowness of the middle meatus. Endoscopic score 0 was normal, 1: mild, 2: moderate, and 3: severe changes of the middle meatus and ostiomeatal complex.

Table 1

Characteristics of patients	Preoperative	Postoperative 9 months
	$n = 30$	$n = 29$
Age, years:		
median	50	
range	21–66	
Number of males (%)	10 (33.3)	10 (34.5)
Number of patients with allergic rhinitis (%)	17 (56.7)	17 (58.6)
Number of patients with asthma (%)	10 (33.3)	10 (34.5)
Number of patients with nasal polyps (%)	0	1 (3.4)
Smokers (%)	8 (26.7)	7 (24.1)
Number of patients without opening of the ethmoid bulla on both sides (%)	5 (16.7)	4 (13.8)
No. of patients using:		
Antihistamine (%)	2 (6.7)	1 (3.4)
intranasal corticosteroids (%)	8 (26.7)	6 (20.7)
Both (%)	4 (13.3)	3 (10.3)

2.4. Symptoms. The symptoms were recorded by a questionnaire preoperatively and at 16 days, 3 and 9 months postoperatively. The following symptoms were asked: facial pain/pressure, nasal obstruction, nasal discharge, postnasal drip, decreased sense of smell, and they were scored: no = 0, mild or moderate = 1, and severe = 2. In addition, lacrimation (none = 0, mild = 1, moderate = 2, and severe = 3) and postoperative bleeding (absent = 0, mild or moderate = 1, and severe = 2) were asked during the debridement follow-up visit at 7–30 days (mean ± SD, 16 ± 5 days), and at nine months postoperatively.

2.5. Computed Tomography Scans. Coronal sinus CT scans were obtained before and 9 months postoperatively. The ostiomeatal complex was reconstructed with 1 mm slice

thickness. LM scores and the area of the ostium were determined, as previously described [13].

2.6. Ethical Considerations. The study was approved by the Institutional review boards of the Tampere University Hospital and Mikkeli Central Hospital. All patients suffered from a moderate-severe form of CRS. Informed consent was obtained from all patients.

2.7. Statistical Analysis. Statistics were performed with SPSS Base 16.0 Statistical Software Package (SPSS, Chicago, IL, USA). Data are expressed as medians and interquartile ranges. The data was tested and found not to be normally distributed. The nonparametric Wilcoxon rank sum test was used for comparison of matched pairs. Kruskal Wallis and Mann-Whitney U tests were used for comparisons of groups. For correlations, the nonparametric Spearman rank correlation test was used. A two-tailed P value of less than 0.05 was considered significant in all tests.

3. Results

3.1. Endoscopic Score. We used the endoscopic score to evaluate semiquantitatively the mucosal status of the operated area. A high endoscopic score indicated swollen or polypous mucosa and/or anatomical narrowness of middle meatal and ostiomeatal complex area. Preoperative observation of both sides of each CRS patient revealed no significant differences statistically in the middle meatal endoscopic scores ($P > 0.05$, by Wilcoxon test, Figure 1). The endoscopic scores did not change significantly between the preoperative, and 16-day and 3-month postoperative periods ($P > 0.05$, by Wilcoxon test, Figure 1). Nor did it change significantly between the 16-day and 9-month periods ($P > 0.05$, by Wilcoxon test, Figure 1). However, when comparing the preoperative endoscopy scores to 9-month postoperative endoscopic scores of each side separately, a significant and identical improvement on both the ostium preserving and enlarging sides was observed ($P = 0.004$, $P = 0.001$, resp., by Wilcoxon test, Figure 1). The endoscopic score was better on the antrostomy side compared to the uncinectomy-only side, but only at 16 days postoperatively ($P = 0.039$, by Wilcoxon test, Figure 1). Interestingly, at 9 months postoperatively there was a correlation between the endoscopic score and the radiologic maxillary sinus LM score (the uncinectomy-only side $P < 0.01$, $R = 0.63$; the antrostomy side $P < 0.05$, $R = 0.51$, by Spearman rank correlation test, Figures 2(a) and 2(b)). Preoperatively, there were no correlations between these variables ($P < 0.05$ by Spearman rank correlation test, data not shown). Allergic rhinitis associated with a higher endoscopic middle meatal score, but only on the ostium-enlarging side and at 9 months postoperatively ($P = 0.037$ by Mann-Whitney U test, data not shown). In contrast, the endoscopic score did not associate with age, sex, smoking, asthma, or medication ($P > 0.05$, by Mann-whitney U and Spearman rank correlation tests, data not shown). Nor did the pre- or post-operative endoscopic scores correlate with any of the symptoms asked at the same time points on either ostium—preserving or-enlarging sides ($P > 0.05$ by Spearman rank correlation test, data not shown).

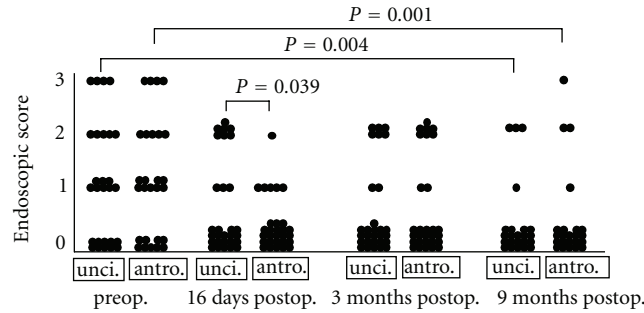

Figure 1: The comparison of endoscopic score between the sides with uncinectomy-only (unci) and additional middle meatal antrostomy (antro) between different time points. The endoscopic score of the middle meatus was semiquantitatively determined: 0: normal, 1: mild, 2: moderate, and 3: severe changes of the middle meatus. P values by Wilcoxon test. Only the P values < 0.05 are shown in the figure.

3.2. Maxillary Sinus Ostium. There were no peri- or postoperative differences between the operation techniques in terms of accessory ostium, endoscopically evaluated ($P > 0.05$, by Wilcoxon test, data not shown). At 16 days postoperatively, eight obstructed maxillary sinus ostia were found on the uncinectomy-only side in contrast to only one on the antrostomy side ($P = 0.031$, by Wilcoxon test, Table 2). Of these, five remained obstructed at also 3 and/or 9 months postoperatively, four on the uncinectomy-only side and one on the antrostomy side (Table 2). At 9 months postoperatively two new ostium obstructions were identified with each technique leading to identical numbers of obstructed ostia between the sides ($P > 0.05$ by Wilcoxon test, Table 2). In one case, the ostium was seen as being obstructed endoscopically but turned out to be widely open on CT-scans taken 9 months postoperatively (Table 2). When dropping out this exceptional case, the ostium findings by CT-scans and by endoscopy were otherwise in line at 9 months postoperatively: the median of radiologic ostium area was higher in cases with endoscopically patent ostium contrasted to those with endoscopically obstructed ostium (the uncinectomy-only side $P = 0.003$, the antrostomy side $P = 0.009$, by Mann-Whitney U test, data not shown). Sex, allergic rhinitis, asthma, smoking, nasal corticosteroids and/or antihistamine use did not associate with an obstructed maxillary sinus ostium ($P > 0.05$, by Fisher test, data not shown). Nor did maxillary sinus obstruction associate to any of the asked symptoms at 9 months postoperatively ($P > 0.05$, by Mann-Whitney U test, data not shown).

3.3. Maxillary Sinus Mucosa. There were no perioperative differences between the sides in terms of maxillary mucosal edema and secretions, endoscopically evaluated ($P > 0.05$, by Wilcoxon test, data not shown). At 9 months postoperatively, there was an insignificant trend that maxillary sinus mucosal

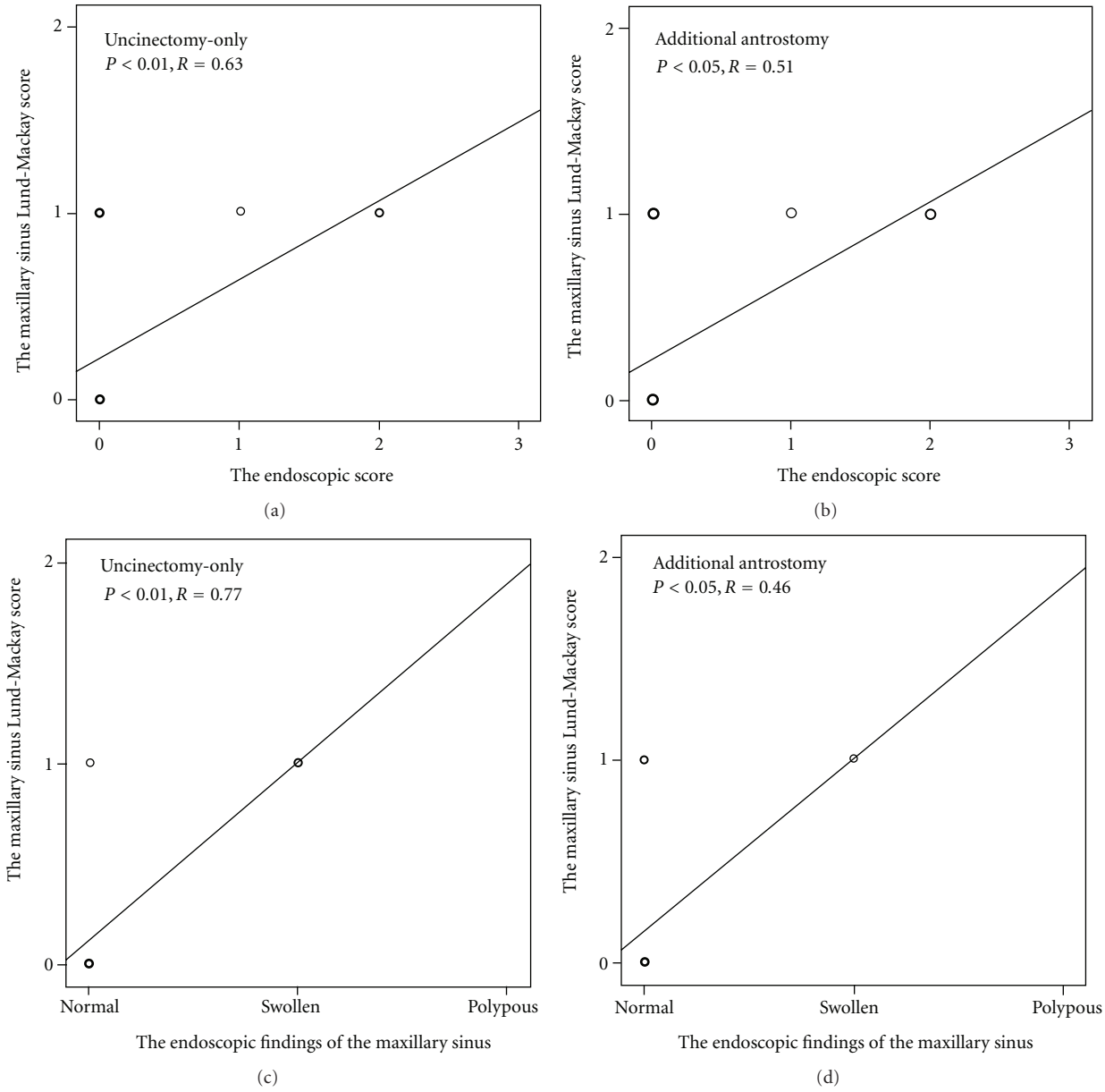

Figure 2: Correlation between maxillary sinus LM-score form CT scans and the endoscopic score of middle meatus on the uncinectomy-only and antrostomy sides at 9 months postoperatively (a) and (b). Correlation between maxillary sinus LM-score and the endoscopic findings of maxillary sinus mucosa on the uncinectomy-only and antrostomy sides at 9 months postoperatively (c) and (d).

edema was more frequently found on the uncinectomy-only side compared to the antrostomy side ($P = 0.083$, by Wilcoxon test, Table 2). Furthermore, at 9 months postoperatively four patients had mucous secretions in the maxillary sinus (Table 2). Although three of these were found on the uncinectomy-side, there were no statistically significant differences between the procedures ($P > 0.05$, by Wilcoxon test, Table 2). None of the patients had pus or polypous mucosa in the maxillary sinus. Endoscopic findings ofmaxillary secretions did not associate with maxillary mucosal edema on either antrostomy or uncinectomy-only sides compared separately ($P > 0.05$, by Fisher test, data not shown). However, there was a correlation between maxillary sinus secretions and endoscopic middle meatal score at 9 months postoperatively (the uncinectomy-only side $P < 0.01$, $R = 0.67$; the antrostomy side $P < 0.01$, $R = 0.58$, by Spearman rank correlation test, data not shown). At nine months postoperatively, endoscopically evaluated maxillary mucosal edema correlated with the radiologic maxillary sinus LM score on both uncinectomy and antrostomy sides ($P < 0.01$, $R = 0.77$; $P < 0.05$, $R = 0.46$, resp., by Spearman rank correlation test, Figures 2(c) and 2(d)). In contrast, a swollen maxillary sinus mucosa or secretions did not associate with any of the symptoms asked postoperatively at the same time points on either ostium-preserving or-enlarging sides ($P < 0.05$, by Spearman rank correlation test,

Table 2: The patients with endoscopic signs of ostium obstruction and/or adhesion formation as complications. Both pre- and postoperative endoscopic scores (0: normal, 1: mild, 2: moderate, and 3: severe changes of middle meatus) are shown. Other endoscopic findings −: no; +: yes; adhesions: ++++: ostium severely restricted, +++: from lateral wall of middle meatus to middle turbinate, ++: from middle turbinate to septum, +: from lower turbinate to septum; [1]patients with missing data, thus the 3-month postoper. data was used, +[2]: the ostium was widely open in sinus CT scans 9 months postoperatively; ?: the maxillary sinus mucosa was not seen; M: male, F: female; d.: days; m.: months. None of the patients suffered from any acute infection, nor had endoscopic signs of polypous maxillary sinus mucosa or pus secretions in maxillary sinus.

Gender		M[1]	M[1]	M	M	M	F[1]	F	F	F	F	F	F	F
Age		46	53	59	63	31	60	22	50	31	49	23	40	65
Allergic rhinitis		+	−	−	−	−	−	+	+	+	+	−	−	−
Asthma		−	−	+	+	+	−	−	−	+	+	−	−	−
Use of intraasal corticosteroids		−	−	−	+	−	−	−	−	+	−	−	+	−
Smoking		−	+	+	−	−	+	−	−	−	+	+	−	−
						Maxillary sinus ostium obstruction								
16 d. postop.	Uncinectomy	+	+	+	+	−	+	−	−	−	+	+	+	−
9 m. postop.	Uncinectomy	−	+	+	−	+	−	−	+	−	−	+	+	−
16 d. postop.	Antrostomy	−	−	−	+	−	−	−	−	−	−	−	−	−
9 m. postop.	Antrostomy	+	−	+[2]	+	+	−	−	−	−	+	−	−	−
						Adhesions								
9 m. postop.	Uncinectomy	+++	−	−	−	+++	+	+++	−	−	−	+++	−	−
9 m. postop.	Antrostomy	−	−	+++	−	+++	−	−	−	+	−	−	−	+++
						Endoscopic score of the middle meatus								
Preoperative	Uncinectomy	0	0	3	0	2	0	1	1	0	3	1	1	1
9 m. postop.	Uncinectomy	0	2	0	1	0	0	0	2	0	0	2	2	0
Preoperative	Antrostomy	0	2	3	0	2	0	1	1	0	3	1	1	1
9 m. postop.	Antrostomy	2	0	3	2	2	0	0	0	0	2	1	0	0
						Swollen maxillary sinus mucosa								
9 m. postop.	Uncinectomy	−	−	?	+	−	−	−	+	+	?	−	+	−
9 m. postop.	Antrostomy	−	−	?	+	−	−	−	−	−	?	−	−	−
						Mucus secretions of maxillary sinus								
9 m. postop.	Uncinectomy	−	−	−	−	+	−	−	+	+	−	−	−	−
9 m. postop.	Antrostomy	−	−	−	−	+	−	−	−	−	−	−	−	−

data not shown). Nor did a swollen maxillary sinus mucosa or secretions associate to age, sex, allergic rhinitis, smoking, asthma, or medication ($P > 0.05$, by Kruskal-Wallis, Mann-Whitney U, and Spearman rank correlation tests, data not shown).

3.4. Adhesion Formation. Preoperatively, there were no signs of adhesions. At 9 months postoperatively, six patients out of 29 had endoscopic findings of adhesion formation; three were on the uncinectomy side and four on the antrostomy side (Table 2). There were no differences between sides in terms of adhesions ($P > 0.05$, by Wilcoxon test, data not shown). Adhesion formation did not associate to sex, allergic rhinitis, smoking, nasal corticosteroids, and/or antihistamine ($P > 0.05$, by Fisher test, data partly shown in Table 2). Nor did the presence of adhesions associate to any of the asked symptoms at 9 months postoperatively ($P > 0.05$, by Mann-Whitney U test, data not shown).

4. Discussion

The important postoperative endoscopic signs are ostium patency, mucosal recovery, and adhesion formation. We found that the side with additional antrostomy showed significantly better early recovery of the middle meatal mucosa and maxillary sinus ostium patency. During nine-month followup, maxillary sinus ostium obstruction was insignificantly more frequently found with the ostium-preserving technique. So were maxillary sinus mucosal swelling and secretions. On the other hand, long-term recovery of the middle meatal mucosa was statistically similarly achieved with both ostium-preserving and ostium-enlarging

procedures. Similarly to our findings, Wadwongtham and Aeumjaturapat. showed in a randomized study that antrostomy was better than uncinectomy early postoperatively, but that at one year postoperatively a 60% rate of patency was achieved with both procedures [14]. One study comparing the antrostomy size, as well as other observational studies, showed good endoscopic recovery after ESS [20, 21].

Our finding that half of early postoperatively obstructed ostia remained obstructed later on with other signs of poor recovery seems to be in accordance with a recent study showing that findings of good recovery even at one month postoperatively seem to predict good long-term results of ESS [22]. On the other hand, previous observations have shown that postoperative mucosal healing takes more than one month [23–26]. Endoscopic parameters, such as middle turbinate position, adhesions, inflammation, and crusting, have shown to have acceptable interexaminer reproducibility and are suitable for evaluating ESS outcomes in the postsurgical period [27, 28].

About two-thirds of patients with recalcitrant CRS might have biofilms in the sinonasal mucosa, but their influence on disease or ESS outcomes have yet to be elucidated [11, 16, 17]. Zhang et al. have observed that bacterial biofilms might associate to asthma and also to adhesion formation and revision ESS [29]. In case of revision sinus surgery, Kennedy has argued in favour of complete uncinate process removal, whilst preserving the mucosa [30]. Wang et al. showed a statistically significant correlation between more advanced bone remodelling and a higher postoperative endoscopic score, thus also reinforcing the putative importance of operating diseased bony/cartilage structures in order to achieve mucosal recovery [31]. In our study, it seems that antrostomy as a more invasive procedure does not cause more frequently adhesion formation.

Patient history factors failed to provide an explanation for the development of adhesions, maxillary ostium obstruction, or mucosal findings. The only exception was allergic rhinitis, which associated with a higher endoscopic middle meatal score, which is not easy to explain as we did not observe increased inferior turbinate swelling in atopic patients pre- or post-operatively [32]. In contrast to what has been found in patients with Samter's triad, allergic rhinitis would not seem to affect CRS severity or outcomes of ESS, as long as atopy is taken into account and treated [33–37]. Tomassen et al. have shown that most patients with CRS symptoms, and also about a third of those without CRS symptoms, have a positive nasal endoscopy [38]. Endoscopic findings did not associate with symptoms in our study, which is in accordance with other observations [20, 39, 40].

The present and previous studies of ours and others show that nasal endoscopy findings correlate strongly with CT-scan scores, thus arguing in favour of radiation reduction by performing proper endoscopy [20, 38, 39]. Still, both CT imaging and endoscopy might be needed, as was shown in a study where CT scans were superior in detecting anatomical variations in all bony or cartilaginous structures, while endoscopy remained superior only in polyp diagnostics [40].

As a methodological shortcoming, with this study setup we were not able to observe the influence of comorbidities on procedure outcomes. On the other hand, the patient was his or her own control which decreases the effect of confounding factors, such as interpatient differences in use of pre-or postoperative medication and early postoperative care.

5. Conclusion

Overall, there was a good and similar long-term endoscopic recovery of the middle meatal and ostiomeatal complex area after maxillary sinus surgery with either the ostium-preserving or-enlarging technique. Antrostomy is, however, better in early middle meatal recovery and ostium patency. This might putatively influence long-term results also. Postoperatively, endoscopy and CT scans provide identical information about the ostiomeatal complex area and maxillary sinus.

Authors' Contribution

All authors contributed equally to this paper. This study was supported by the ethical committees of the Tampere University Hospital and Mikkeli Central Hospital.

Conflict of Interests

The authors have no conflict of interests to declare.

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