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NASAL POLYPOID RHINOSINUSITIS - CLINICAL COURSE AND ETIOLOGICAL INVESTIGATIONS

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Academic Dissertation

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Yliopistopaino

“Omnia tempus habent”

To my husband Antti and children Kreetta and Juho

CONTENTS

ABSTRACT

Background: The etiology and pathology of nasal polyposis are mainly unknown. It seems to be partially related to infection but it is mainly related to eosinophilic inflammatory mechanism as in acetylsalicylic acid (ASA) intolerance or asthma. The purpose of the present study was 1) to deepen the understanding of the clinical course of nasal polyposis over a long period of time, 2) to examine the reliability of A-mode ultrasound (A-US) in detecting fluid retention or mucosal thickening in patients with chronic polypoid sinusitis, 3) to analyse the sense of smell in patients with long-standing nasal polyposis as compared to a healthy control material, 4) to investigate the occurrence of VPF/VEGF in nasal polyps of patients with chronic nasal polyposis for two decades as compared to normal nasal mucosa and 5) to find out the frequency of the two most common CF mutations in patients with chronic sinusitis with or without nasal polyps.

Materials and methods: Studies I-IV were based on a series of 109 patients who underwent polypectomy at the Department of Otorhinolaryngology, University Central Hospital, Helsinki in 1977-78. The patients were then divided into three groups (ASA intolerance, atopic (AT) and intrinsic allergy-like disease (INTR)) according to the results of clinical and allergological investigations. Forty-one of those 109 patients were after about twenty years re-evaluated and classified as earlier into three groups and examined as follows: physical examination, sinus computed tomography (CT), A-US, olfactory threshold measurement, odour detection test, skin prick tests, nasal smears, active anterior rhinomanometry, acoustic rhinometry and a biopsy from a polyp or from the mucosa of the middle turbinate, if no polyp was seen. Information on nasal symptoms, present and past history of nasal disorders was gathered by means of a questionnaire completed by all subjects. A-US examination (done twice by two investigators) of the maxillary sinus and sinus CT were done in the course of 2 hours. The two investigators had no knowledge of patient history, CT findings or the other investigator's results. The ultrasound findings recorded by investigator 1 were carefully analysed for reliability by comparison with CT evidence. The results obtained by investigator 2 were used to assess the reproducibility of A-US

diagnoses. Vascular permeability/vascular endothelial growth factor (VPF/VEGF) expression was determined using a mouse monoclonal antihuman VPF/VEGF antibody. In study V the determination of cystic fibrosis gene mutation carriers (394delTT and F508) were done in 127 patients with chronic sinusitis. Genomic DNA was isolated from the whole blood specimens according to standard protocols. Positive controls from CF patients were used.

Results: Sixty-eight percent of 41 patients thought that during 20 years the activity of their disease had decreased or the disease had resolved. However polyps could be seen in anterior rhinoscopy in 85% of the patients. The patients with ASA intolerance had a higher recurrence frequency, a greater need for surgical treatment than the patients in the AT and INTR groups. The sensitivity of A-US in detecting fluid or total obliteration was 30% and the specificity 81%. In detecting mucosal thickening the sensitivity of A-US was 40% and the specificity 48%. The two investigators obtained identical A-US results in only 50%. Forty-six percent of patients had anosmia or hyposmia. The association between total obliteration of both olfactory recesses and olfactory thresholds was statistically significant. In the forward stepwise multiple linear regression analysis of all patients, the degree of obliteration of ethmoidal sinuses in CT, polyposis seen in anterior rhinoscopy, the logarithmically transformed total nasal resistance and gender showed significant association with olfactory thresholds. The staining for VPF/VEGF in the mucosal surface and in the glandular epithelium of nasal polyps was weaker than in normal controls. In two patients strong staining for VPF/VEGF was found in granules of mast cells. One carrier of the 394delTT mutation was found among 127 chronic sinusitis patients.

Conclusion: This study shows that the activity of nasal polyposis does not cease in the course of 20 years. Although A-US can be a valuable investigation method in a simple acute sinusitis, it should not be used in patients with chronic sinusitis history, polyposis and especially in transantrally operated maxillary sinuses. Chronic nasal polyposis diminishes the sense of smell. The expression of VPF/VEGF seems to be weaker in polyps than in normal nasal mucosa but strong staining of VPF/VEGF in mast cells needs further study to find out its meaning. Routine screening of sinusitis patients for CF mutations gives no more information on the etiology of chronic sinusitis in Finnish population.

ABBREVIATIONS

AFS = allergic fungal sinusitis
ALU = arbitrary logarithmic unit
AMP = adenosine monophosphate
ANOVA = analysis of variance
ASA = acetylsalicylic acid
AT = atopic
A-US = A-mode ultrasound
bFGF = basic fibroblast growth factor
B-US = B-mode ultrasound
CF = cystic fibrosis
CFGAC = the Cystic Fibrosis Genetic Analysis Consortium
CFTR = cystic fibrosis transmembrane conductance regulator
CT = computed tomography
DNA = deoxyribonucleic acid
DS = decismel
ECP = eosinophil cationic protein
ELISA = enzyme-linked immunosorbent assay
ENT = Ear, Nose and Throat
EPO = eosinophil peroxidase
flt-1 = fms-like-tyrosine kinase-1
Ig = immunoglobulin
IGF-I = insulinlike growth factor I
IL = interleukin
INTR = intrinsic allergy-like disease
KDR = kinase domain region
LT = leukotriene
MBP = major basic protein
MRI = magnetic resonance imaging
mRNA = messenger ribonucleic acid
NARES = nonallergic rhinitis with eosinophilia syndrome
PAF = platelet activating factor
PCD = primary ciliary dyskinesia
PCR = polymerase chain reaction
PG = prostaglandin
RANTES = regulated upon activation, normal T-cell expressed and secreted

ABBREVIATIONS

SD = standard deviation

US = ultrasound
UPSIT = the University of Pennsylvania Smell Identification Test
VCAM-1 = vascular cell adhesion molecule 1
VPF/VEGF = vascular permeability/vascular endothelial growth factor

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which will be referred to the text using the roman numerals I to V.

I Vento SI, Ertama L, Hytönen M, Wolff H, Malmberg H. Nasal polyposis: clinical course during 20 years. Ann Allergy Asthma Immunol 2000; 85: 209-14.

II Vento SI, Ertama L, Hytönen M, Malmberg H. A-mode ultrasound in the diagnosis of chronic polypous sinusitis. Acta Otolaryngol (Stockh) 1999; 119: 916-20.

III Vento SI, Simola M, Ertama LO, Malmberg CHO. Sense of smell in long-standing nasal polyposis. Am J Rhinol. In press.

IV Vento SI, Wolff H, Salven P, Hytönen M, Ertama L, Malmberg H. Vascular permeability factor/vascular endothelial growth factor in nasal polyps. Acta Otolaryngol, 2000; Suppl 543: 162-4.

V Hytönen ML, Patjas MT, Vento SI, Kauppi PM, Malmberg H, Ylikoski JS, Kere JK. Cystic fibrosis gene mutations F508 and 394delTT in patients with chronic sinusitis in Finland. Submitted.

Some unpublished data have also been included.

1. INTRODUCTION

The history of nasal polyposis goes back nearly 5000 years to Ancient Egypt, although the exact details are obscure (Brain 1997). The prevalence of nasal polyps is reported as 1-2% of the adult population in Europe (Hosemann et al. 1994) and 4.3% in Finland (Hedman et al. 1999). The predominance of males over females is a ratio of 2-4:1 (Drake Lee 1987).

Several conditions and symptoms are related to the occurrence of nasal polyps. In asthmatic patients occurrence rates from 7% to 42% have been reported (Weille 1936, Settipane and Chafee 1977, Maran and Lund 1990). Nasal polyps are common in patients with acetylsalicylic acid (ASA) intolerance, the frequency going up 40% to 60% of these patients (Maran and Lund 1990). A comorbid disease with nasal polyposis is cystic fibrosis (CF), which is one of the most frequent hereditary diseases found in Caucasians. Polyps are found in adult patients with CF in 20-48% (di Sant' Agnese and Davis 1979, Aitken and Fiel 1993). Nasal polyps are also strongly associated with chronic rhinosinusitis of different origin (Maran and Lund 1990).

Nasal polyps are usually bilateral, multiple, and freely movable smooth tumours. Clinical symptoms are obstruction of the airflow through the nose, often hyp- or anosmia, postnasal dripping and nasal secretion. Systemic or intranasal steroids are commonly used in their treatment, but in cases of serious blockage or infectious complications also surgery is indicated. The recurrence of nasal polyposis constitutes a serious clinical problem. Recurrence rates up to 29-53% have been reported (Blumstein and Tuft 1957, Jäntti-Alanko et al. 1985, Larsen and Tos 1994). Nasal polyposis can be a frustrating disease for the patient and for the treating physician.

The etiology and pathophysiology of nasal polyposis are still mainly unknown though significant knowledge has been obtained about nasal physiology and nasal polyposis with scientific advances in the fields of biochemistry, microbiology, and immunology. In recent years, several studies have drawn attention to e.g. growth factors in nasal polyps.

Before effective prevention and treatment of the disease can be achieved, more knowledge is needed about nasal polyposis.

2. REVIEW OF THE LITERATURE

2.1. HISTORY OF NASAL POLYPS

The history of nasal polyps goes back over 4000 years to Ancient Egypt. Further significant advances were made in Ancient Greece and Renaissance Europe. The first known medical practitioner was an Egyptian rhinologist called Ni-Ankh Sekhmet who was the Court Physician to King Sahura and his picture together with that of his wife were found on a slab in the tomb of the king together with a testimony of royal gratitude which states ”he has made his nostrils well” (Brain 1997). It has been shown that the Egyptians were familiar with nasal polyps, which they described as ”grapes coming down from the nose”. Treatment included medicaments containing alcohol and it is possible that some of the Egyptian surgical instruments may well have been used to remove polyps (Pahor and Kimura 1991). Hippocrates (460-370 B.C.), the ”Father of Medicine” thought that polyp disease resulted from a disturbed equilibrium between the 4 ”humours”. When the humours were too thick it could result in the development of polyps (Brain 1997). He developed a technique for removal of polyps, which was included in textbooks as late as that of Voltolini’s in 1888 (Vancil 1969). This method was the ”sponge” method in which the ends of three or four strings were tied to a sponge cut to the proper size and shape. The other ends were knotted together and fastened to the eye of a malleable tin or lead probe, which was then passed through the nose and into the pharynx. The secured ends of the strings were passed over the end of a forked probe held in the pharynx. The polyps were delivered with the sponge with a successful manoeuvre. For harder growths, he used the described technique, but he substituted a loop of sinew for the sponge and adjusted it around the polyp before applying traction. He also used cauterisation with hot iron through a hollow tube serving as a speculum. Polypectomy was followed by the local application of copperas caustic powder and the insertion of small lead plates smeared with oil and honey (Vancil 1969).

Paulus of Aegina (625-690 A.D.), a physician of high reputation in Alexandria, believed that the ethmoid cells were the origin of nasal

polyps. He devised a rather barbarous method for polyp removal. ”Taking, then, a thread moderately thick, like a cord, and having tied knots upon it at the distance of two or three finger breadths, we introduce it into the opening of a double-headed speculum (probe) and we push the other extremity of the speculum upward to the ethmoid openings passing it by the palate and mouth; and then, drawing it by two hands, we saw away, as it were, with the knots, the fleshy bodies. After the operation, we keep the opening separate by means of a tent resembling the wick of a lamp.” Postoperatively he sponged the parts carefully and injected oxycrate of wine into the nose. If fluids descended by the roof of the mouth to the pharynx, the operation had succeeded (Vancil 1969).

In the era of Arabian Medicine Avicenna (980-1037 A.D.) described nasal polyps as ”piles in the nose” or haemorrhoids” in the nose. Abulcasis (1013-1106 A.D.), the greatest of the Arab surgeons used cautery and pulled the nasal polyp forward with a hook, cut through the pedicle with scissors and then washed the nasal cavity with vinegar (Pahor and Kimura 1991).

In the Middle Ages at the 11th and 12th century, the School of Salerno flourished and Greek and Arabian medical books were translated into Latin. Garlic was inserted into the nose after being wrapped in cloth leading to sneezing and ”expulsion” of the polyps. Polyps were removed with knives, hot rods and forceps (Pahor and Kimura 1991).

In Renaissance Forestus (1522-1597 A.D.) recorded a case history which showed that the theory of 4 ”humours” did linger on to much more recent times. (Pahor and Kimura 1991, Brain 1997). In the case of anosmia he stated that if it is from ethmoidal obstruction, or from the humour discharged from a catarrh, the latter must first be cured (Vancil 1969). Forestus described a case of a woman in whose nostrils a huge polyp had grown due to her carrying heavy weights on her head, which forced the mucus down into the membranes of her nose. She was cured by ligation of the polyp and the application of vitriol to its stump but it recurred when the woman resumed her occupation and it was again cured in the same way (Brain 1997).

Fallopius (1523-1562 A.D.) used ligatures, leaving them around the anterior polyps for two or three days, when they would fall off with the constricted mass. For posterior polyps, which he considered as

haemorrhoids, he designed the first major advancement in the principle of the snare, since Hippocrates. The same instruments he also used for rectal polyps (Vancil 1969).

The operations were in fact unsatisfactory because they were always painful, and potentially dangerous because the surgeon nearly always had a lack of visual control. Further impetus was gained when Carl Koller, in 1884, discovered the surgical possibilities of cocaine (Vancil 1969). Nasal polypectomy did not become a routine minor operation until the end of the 19th century (Brain 1997). As early as 1901 Hirschman used a 4 mm cystoscope to get a better view into the nasal cavities and nasopharynx (Hirschman 1903) and in the late 1960's and early 1970's Messerklinger from Graz in Austria developed a technique of systematic endoscopic investigation (Stammberger 1997). Nowadays nasal snares are improved in design and we have endoscopes, shavers, silver nitrate sticks, electro-cautery and many types of nasal decongestants and steroids at our disposal.

2.2. EPIDEMIOLOGY

2.2.1. The prevalence of nasal polyps

The prevalence of nasal polyps is reported as 1-2% of the adult population in Europe (Hosemann et al. 1994) and 4.3% in Finland (Hedman et al. 1999). The prevalence of nasal polyposis has been estimated to be about 4% in patient referrals to allergy clinics in USA but it has been reported to be much higher in selected populations (Settipane and Chaffee 1977, Maran and Lund 1990). In a study on cadavers, 42% were found to have nasal polyps (Larsen and Tos 1996). The predominance of males over females is with ratio of 2-4:1 (Drake Lee 1987). The manifestation of nasal polyps is usually after the age of 20 years. Initial presentation occurs then equally in each decade up to 60 years, but after this they are encountered more rarely. They are also considered to become less troublesome as the patient ages (Drake-Lee 1994). Nasal polyps in children are very rare – an incidence of 0.1% has been reported (Settipane and Chafee 1977). Nasal polyps are rare in animals and the chimpanzee is the only animal known to suffer from a similar polyposis as humans (Drake-Lee 1987).

2.2.2. Nasal polyps and CF

Long known to be an autosomal-recessive illness, CF was found in 1989 to be caused by a mutation of a normal gene on the long arm of chromosome 7. The gene is known as the cystic fibrosis transmembrane conductance regulator (CFTR) gene (Kerem et al. 1989, Riordan et al. 1989, Rommens et al. 1989). The CFTR gene codes for a protein that functions as a cyclic adenosine monophosphate (AMP)-regulated chloride channel. CFTR protein is expressed on the lumenal surface of pancreatic and sweat gland epithelial cells and in submucosal glands and epithelium lining the airways (Deane and Schwartz 1997). To date, over 900 gene mutations causing CF has been identified (The Cystic Fibrosis Genetic Analysis Consortium, CFGAC: http://www.genet.sickkids.on.ca/cftr). CF patients have abnormal sodium and chloride transport mechanisms causing disturbances in the mucus production forming and mucus transporting on the ciliated respiratory epithelium. A dehydrated thickened viscous mucus impairs mucociliary clearance (Deane and Schwartz 1997). The typical clinical features for CF patients are an abnormally high concentration of chloride in the sweat, lung and paranasal infections, nasal polyposis, pancreatic insufficiency, male infertility and decreased female fertility (Aitken and Fiel 1993). As many as 10% of children with CF may have concomitant nasal polyps (Maran and Lund 1990). Adult patients with CF have even higher frequencies of nasal polyps, reported to be 20-48% (di Sant' Agnese and Davis 1979, Aitken and Fiel 1993).

2.2.3. Nasal polyps, asthma and ASA intolerance

Although nasal polyposis seems to be partially related to infection (characterised by neutrophilic-lymfocytic inflammation), it is mainly related to the eosinophilic inflammatory mechanism as in ASA intolerance or asthma (Holopainen et al. 1979, Settipane 1996). Asthma is found in about one-third of adults with polyps (Drake-Lee et al. 1984b) and 7% of patients with asthma have nasal polyps (Settipane and Chafee 1977).

Some 15-36% of patients with nasal polyps suffer from ASA intolerance (Chafee and Settipane 1974, Schapowal et al. 1995) and up

to 60% of patients with ASA intolerance have nasal polyps (Maran and Lund 1990). The triad of nasal polyps, ASA intolerance and asthma was first described by Widal, Abrami and Lermoyez in 1922. This association was later emphasised by Samter (Samter and Beers 1967). The classic triad occurs in approximately 8-39% of all polyp cases (Drake-Lee 1987, Maran and Lund 1990).

In Finland the prevalence of nasal polyposis is 16.5% in asthmatic patients and the prevalence of the triad nasal polyposis, ASA intolerance and asthma is 4.3% in patients with asthma (Hedman et al. 1999). A hereditary factor has been considered to be involved in the development of nasal polyps (Settipane and Pudupakkam 1975, Greisner and Settipane 1996).

2.3. CLINICAL PICTURE AND DIAGNOSIS

2.3.1. Clinical symptoms and complications

Nasal polyps are usually bilateral multiple and freely movable protrusions of benign oedematous mucosa. They are glistening, pale-grey smooth and semitranslucent in appearance. Polypoid lesions are mainly situated in the middle meatus and most of the polyps originate from the mucosa of the ostia, anterior and posterior ethmoidal clefts and frontal and sphenoethmoidal recesses but also from middle turbinate and septum (Larsen and Tos 1991, Larsen and Tos 1996). Polyps may occur in all paranasal sinuses, too (Drake-Lee 1987). Polyps visible in the middle meatus usually originate from the mucosa of the ostia, anterior ethmoidal cleft or frontal recess but polyps visible between the nasal septum and the middle or the superior turbinates either originate from the olfactory ridge, posterior ethmoidal cleft or sphenoethmoidal recess (Larsen and Tos 1991, Larsen and Tos 1996, Stammberger 1997). Antrochoanal polyps arise in the maxillary antrum and prolapse through the ostium of the sinus in the middle meatus. They are seen in the nose or, if larger, in the posterior choana (Drake-Lee 1987). Most frequently nasal polyps are found on the concave site of the deviated septum (Maran and Lund 1990).

Clinically nasal polyposis is characterised by oedematous masses in the nasal and paranasal cavities, leading to nasal obstruction, retention of nasal and sinus secretion, loss of smell, headache, and reduced

general well-being (Bachert et al. 1998). When nasal obstruction becomes total, the patient gets symptoms of secondary sinusitis, and even anatomical changes, with a widening of the bridge of the nose. Over 80% have secondary sinusitis and with it, there may be an associated cough and bouts of acute sinusitis. (Maran and Lund 1990). The relationship between polyps and sinusitis is critical and the presence of inflammation may be a strong trigger for polyp disease. Nasal polyps can produce a wide range of radiological changes. The commonest is generalised bilateral loss of translucence in all sinuses, but in addition, 20% of patients show evidence of ethmoidal expansion called the ”remodelling”- phenomenon (Maran and Lund 1990, Som et al. 1991).

In aggressive paranasal and nasal polyposis, erosion of ethmoid bones, walls of the sphenoid sinuses and the floor of the anterior cranial fossa and other sinus walls can happen resulting in consequent ophthalmologic and neurologic sequeale even blindness (Winestock et al. 1978, Rejowski et al. 1982, Som et al. 1991). It has been suggested that probably obstruction of the ostia by polypoid degeneration can also result in a mucocele (Finn et al. 1981), which is an encapsulated mucous-filled mass lined with a low columnar or cuboidal epithelium, containing occasional goblet cells. The mucoperiosteal lining of the sinus becomes widened, with dense connective tissue of varying thickness supporting the epithelial lining (Schuknecht and Lindsay 1949).

2.3.1.1. Recurrence of nasal polyps

There are few reports on the clinical course and recurrence of nasal polyps. In the study published by Blumstein and Tuft (1957) recurrence of polyposis after repolypectomy and allergy management was found in 53% of cases during a follow-up for 4.3 years. Some of those patients with recurrent polyps were followed for 5 to 7 years. This follow-up study showed that the patients with the highest recurrence of nasal polyps had a high incidence of asthma and no specific etiology with negative skin reactions while the patients with identifiable and controllable allergies had the lowest recurrence of polyps. Larsen and Tos described the clinical course of primary nasal polyposis in 1994, the follow-up time being 1 to 8 years (median 57 months). They found that patients with asthma, acute recurrent or

chronic sinusitis, ASA intolerance or atopic allergy required more polypectomies and more topical steroid treatment than patients without these conditions. In another study of nasal polyp patients followed for approximately 8 years (38-145 months) after simple polypectomies Larsen and Tos (1997) found that ASA intolerant patients had the highest number of polypectomies and patients with asthma had more polypectomies than those without them. Jäntti-Alanko et al. (1985) described a 4-year follow-up study of patients with nasal polyps, and in that study the frequency of re-operations was significantly higher and the use of topical corticosteroid treatment more frequent among patients with ASA intolerance than patients without ASA-intolerance. It is commonly thought that recurrence and activity of nasal polyposis diminish with time but studies with long enough follow-up periods to confirm this have not been published.

2.3.1.2. Loss of the sense of smell

Diminished olfactory function is a common complaint in patients with nasal polyposis. The disorders of olfaction have been described as conductive or sensorineural. (Ophir et al. 1986). A diminished sense of smell associated with chronic rhinosinusitis with or without nasal polyposis is thought to be predominantly conductive, a result of mucosal obstruction to airflow through the superior nasal meatus (Hosemann et al. 1993). It has been shown that increases in the size of compartments of the nasal cavity around the olfactory cleft generally increase olfactory ability (Hornung and Leopold 1999). Also the association between nasal allergy and a loss or diminution of smell has been noted frequently in the literature (Fein et al. 1966, Cowart et al. 1993, Apter et al. 1995). Olfactory thresholds are also known to be related to age being markedly higher among elderly people (Doty et al. 1984, Simola and Malmberg 1998), which must be taken into consideration when studying sense of smell among elderly patients.

It has been shown that past history of recurrent polyp operations is associated with poorer smell test results in chronic rhinitis patients (Simola and Malmberg 1998). Hosemann et al. (1993) found that 87% of those patients for whom ethmoidal surgery did not improve the hyposmia or anosmia had already earlier had surgical interventions to their paranasal sinuses.

Olfactory disturbances occur also in nasal infections, sometimes even irreversible anosmia can follow. It has been shown in a biopsy study, that chronic nasal infection may damage areas of the olfactory organ and this will be replaced with respiratory epithelium (Douek et al. 1975). In a study of Kern (2000) 30 patients underwent olfactory biopsy at the time of surgery with evaluation by a pathologist. Nineteen of these 30 patients had olfactory mucosa in the biopsy specimen, the rest had only respiratory or indeterminate mucosa. Ten patients had pathological changes in the olfactory mucosa with an influx of lymphocytes, macrophages, and eosinophils and 7 of these ten had olfactory deficits as determined by the University of Pennsylvania Smell Identification Test (UPSIT). Nine patients had normal olfactory mucosa and normal olfactory function. Infections are usual complications in chronic polyposis, and some effects on the olfactory sense may also be due to these kinds of sensorineural mechanisms. However the methods used for smell identification for measuring olfactory threshold do not distinguish between a sensorineural and a conductive defect.

2.3.2. The diagnosis of nasal polyps

2.3.2.1. Clinical examination

The diagnosis of massive intranasal polyposis associated with subjective nasal congestion is not difficult. Simple anterior rhinoscopy reveals such polyps easily. Antrochoanal polyps can also been seen in posterior rhinoscopy. Smaller polyps limited to the middle meatus including ethmoidal bulla, frontal recess, and uncinate process are more difficult to see, but they can often reliably be identified endoscopically (Penttilä et al. 1990).

2.3.2.2 Imaging techniques

Polypoid masses in the paranasal sinuses can be diagnosed by x-ray, sinus computed tomography (CT) scanning, magnetic resonance imaging (MRI) or ultrasound (US). The commonest finding in x-ray is generalised bilateral loss of translucence in all sinuses (Maran and Lund 1990). It is not radiologically possible to establish the true nature of an opaque sinus, i.e., if it is a fluid, (polypoid) mucous membrane

thickening, cyst or tumour. Axelsson et al. (1970) demonstrated secretions by irrigation in 86% of the completely opaque maxillary sinuses in x-ray and in 60% of cases with mucous membrane thickening. In the latter cases discharge was demonstrated by x-ray in 24% when four standard projections were used.

A-mode ultrasound (A-US) is a simple, non-invasive and non-ionising method for detecting fluid or even mucosal swelling in inflamed maxillary and frontal sinuses. A-US is based on the principle that reflection of US occurs at the boundary of two media with differing acoustic impedances (acoustic impedance = density of the medium x sound velocity in the medium). The boundary of two soft tissues reflects less than 10%, the boundary between soft tissue and bone reflects 50-70% and air acts as a total reflector. A sinus filled with secretion or a cyst or a loose polypoid mucosal thickenings causes a strong echo from the bony back wall (Revonta 1980).

To avoid repeated exposure to ionising radiation, A-US is widely used in diagnosing sinusitis in health centres and hospitals in the Scandinavian countries and in Finnish primary care. A-US has been shown to be quite reliable in the diagnosis of acute maxillary sinusitis with fluid retention (Mann et al. 1977, Revonta 1979, Puhakka et al. 2000) but controversy still exists over the reliability of A-US in detecting fluid retention or mucosal swelling in patients suffering from chronic rhinosinusitis (Mann et al. 1977, Druce et al. 1988) and in detecting mucosal swelling or polyps/cysts in patients with acute maxillary sinusitis (Revonta 1980, Jensen and von Sydow 1987). Slight or moderate mucosal thickenings have been distinguishable from retained secretion, but large cysts/polypoid swellings of the mucosa produced a finding indistinguishable from retained secretion in a study by Revonta (1980). There are however studies with poor correlation. Using A-US and x-ray to evaluate antral mucosal thickening in chronic sinusitis, Druce et al. (1988) found the total specificity to be 61% and the sensitivity 34%. In a study by Jensen and von Sydow (1987) the A-US findings of mucosal thickening without fluid correlated with x-ray in only 19% and different degrees of mucosal thickening were poorly demonstrable. These contradictory A-US results indicate that further studies are needed. There are probably patients in whom A-US is not a suitable tool for diagnosing fluid level or mucosal thickening.

The A-mode technique is increasingly being substituted by B-mode ultrasound (B-US) used by radiologists. B-US provides two-dimensional information about the structures examined, but there are few studies concerning B-US and paranasal sinuses. In a study by Tiejden et al. (1998) 78 patients were examined by CT and subsequently by B-US, two-thirds before endonasal surgery and one-third for diagnosis of serious facial pain and swelling. Eighty-three of 114 pathological maxillary sinus findings in CT could also be diagnosed by B-US (sensitivity 72.8%). Except for circumscribed polyps and moderate general swelling of the mucosa, the detection rate by US sonography was 97.4% for maxillary sinuses.

Sinus CT is currently the modality of ionising choice in the evaluation of the paranasal sinuses and adjacent structures. Its ability to optimally display bone, soft tissue, and air facilitates accurate depiction of the anatomy and extent of disease in and around the paranasal sinuses (Zinreich 1990, Zinreich 1992).

Non-ionising MRI can be also used but it is still often unattainable for economic reasons. MRI has superseded CT in resolving soft tissue structures and is superior in distinguishing between bacterial and fungal sinusitis as well as inflammation and neoplasm, its use for the evaluation of nasal anatomy, particularly for the ethmoid sinuses, is precluded because of its poor resolution of bone (Zinreich 1992).

2.3.3. Histological findings

Nasal polyps are characterised by a pseudostratified ciliated columnar epithelium, thickening of the epithelial basement membrane, few blood vessels and nerve endings (Frenkiel and Small 1991). In general, the stroma of nasal polyps is oedematous, which may be the result of a leakage of plasma through open endothelial junctions in the blood vessels (Cauna 1972). The stroma regularly contains eosinophils, neutrophils, lymphocytes, monocytes, plasma cells, mast cells and macrophages. The vascularisation is poor and lacks vasoconstrictor innervation, with the exception of the nerve terminals in the pedicle of polyp. Mast cells occur about twice as frequently in nasal polyps as in the normal nasal respiratory mucosa and the mast cell granules of polyps are relatively small and they either lack the outer lamellar element or are largely degranulated (Cauna 1972). Tissue eosinophilia

is a general character of nasal polyps and is found in 80-90% of all cases (Settipane 1996, Drake-Lee 1987). In antrochoanal polyps eosinophilia can be found in 20-65% of cases (Heck et al. 1950, Min et al. 1995). Mucus glands almost never can be found in antrochoanal polyps but frequently in nasal polyps (Heck et al. 1950). One way to classify polyps is a subdivision into four histological different types according Hellquist, which sometimes can be essential due to the differential diagnosis of neoplastic disease (Hellquist 1996).

2.3.3.1. Oedematous, eosinophilic polyp

The predominant histological finding is an oedematous, eosinophilic type of inflammation, with an incidence rate of 86% of sinonasal polyps (Davidsson and Hellquist 1993). In this kind of polyp the histological hallmarks comprise oedematous stroma, often hyperplasia of the seromucous glands, numerous eosinophils and mast cells and thickened basement membrane (Hellquist 1996).

2.3.3.2. Other types of polyps

For the chronic inflammatory polyp the lack of stromal oedema and goblet cell hyperplasia are the most striking features. Goblet cells are present but the epithelium is devoid of goblet cell hyperplasia. The epithelium frequently shows squamous and cuboidal metaplasia (Hellquist 1996). This fibro-inflammatory neutrophil type represents less than 10% of sinonasal polyps (Davidsson and Hellquist 1993). This histological type is often found in CF, primary ciliary dyskinesia (PCD) syndrome or Young's syndrome (Settipane 1996).

The third type of polyp is a polyp with hyperplasia of seromucinous glands (Hellquist 1996). This polyp type can be misdiagnosed as an adenoma (Friedmann and Osborn 1982). This type of nasal polyp is rare and represents less than 5% of sinonasal polyps (Davidsson and Hellquist 1993).

The fourth type polyp with stromal atypia is very rare and it has distinct histological features. The main feature, which distinguishes a polyp with atypical stroma from a neoplasm, is a lack of mitoses (Hellquist 1996).

2.3.4. Differential diagnosis

Initially polyps were thought to be of neoplastic origin (Drake-Lee 1987). In the presence of nasal polyps other considerations should also taken into account, namely tumours (e.g. inverted papilloma, juvenile nasopharyngeal angiofibroma, esthesioneuroblastoma, carcinoma, sarcoma, melanoma, meningioma and glioma (in the newborn)) and meningoencephalocele especially in children.

2.4. ETIOLOGY OF NASAL POLYPS

The etiology of nasal polyposis is mainly unknown although it has been connected with many disease states. Different inflammatory factors, e.g. viral upper respiratory infections, allergic rhinitis and various functional disturbances of the mucous membrane have been reported to be predisposing factors leading to sinusitis (Gungor and Corey 1997).

2.4. 1. Allergy

Because of the high concentration of eosinophils within polyps, the popular concept was that all nasal polyps were associated with allergies in the 1930s (Kern and Schenk 1933). Many attempts were made to link nasal polyps to allergens, but this concept was rejected by most physicians in the 1970s and 1980s when investigators were unable to demonstrate an allergy linkage. Numerous publications have shown that there is no higher incidence of allergies and no elevated rates of positive allergy skin tests in the patients with nasal polyps (Caplin et al. 1971, Settipane and Chafee 1977, Perkins et al. 1989). However even in recent years an immunoglobulin E (IgE)-mediated mechanism and elevated incidences of allergies have been reported for a small subgroup of patients with nasal polyps (Sin et al. 1997).

2.4.2. Infections

Many patients with nasal polyps have secondary chronic sinusitis (Maran and Lund 1990). Blockage of the sinus ostia appears to begin the series of events leading to chronic sinusitis (Gwaltney et al. 1995).

2.4.2.1. Virus infection

Weille (1966) suggested that polyps are caused by viral infection. Kozak et al. (1991) had a hypothesis for the viral etiology of nasal polyps including the following steps: viral infection, inflammatory response, persistence of the virus, persistent antigenic stimulation, nasal polyposis. They studied the role of viruses by hybridisation tests for deoxyribonucleic acid (DNA) of some infectious agents in nasal polyps but the tests were negative. They stated that the absence of virus in the tissue supported their hypothesis that viral involvement in nasal polyps is an early event.

2.4.2.2. Bacterial infection

Chronic sinusitis is a common disease with patients of nasal polyposis and thus purulent infection might be an important factor in the etiology of nasal polyps. In rabbits polyps have been shown to develop in the sinus mucosa in three weeks after artificial infection by pneumococci. No polyps developed on the contralateral, non-infected maxillary sinus in those 6 animals (Norlander et al. 1993). Polyps can then have an inflammatory origin with the predominant cells being lymphocytes and neutrophils. Antrochoanal polyps have been associated with inflammatory disease (Min et al. 1995). Many kinds of diseases also can contribute to this situation. In these cases the diagnoses to be considered are CF and PCD syndrome. However these diseases are not primarily infective; in these conditions, infection arises secondary to the defect in the mucosal defence (Mackay and Cole 1987).

2.4.2.3. Fungal infection

Allergic fungal sinusitis (AFS) is a newly appreciated diagnosis. In 1971, McCarthy and Pepys observed that 10% of patients with allergic bronchopulmonary aspergillosis complained of producing nasal plugs similar to those expectorated from the bronchi. In that study 41.4% (46/111) had maxillary sinusitis as shown on x-ray films. Aspergillus fumigatus grew in cultures of the nasal plug and sinus lavages. Katzenstein et al. (1983) described the case histories of seven otherwise healthy patients with asthma, nasal polyposis, sinusitis and a unique ”allergic mucin” within sinuses. This allergic mucin contained laminated mucin, eosinophils, Charcot-Leyden crystals, and fungal hyphae. This condition was called ”allergic aspergillus sinusitis”. With heightened awareness of the disease, an increased number of reports have been published recently. The common diagnostic criteria for allergic fungal sinusitis are (1) chronic rhinosinusitis, (2) the presence of allergic mucin (clusters of eosinophils and their by-products, e.g., Charcot-Leyden crystals and major basic protein (MBP)), and (3) the presence of fungal organisms within that mucin, confirmed by histology, culture or both (Cody et al 1994, deShazo and Swain 1995, Ramadan and Quraishi 1997). In a study of 210 consecutive chronic rhinosinusitis patients with or without polyps, fungal cultures of nasal secretions were positive in 202 (96%) patients and in 100% (14/14) of control patients (Ponikau et al. 1999). In that study during the culturing of nasal secretions, fungi had to be extracted from the mucus before being placed on the growth medium. The minimum incubation time of 30 days seemed necessary for complete recovery. They proposed the term eosinophilic fungal rhinosinusitis instead of AFS because IgE-mediated hypersensitivity to fungal allergens was not evident in the majority of AFS patients.

2.4.3. Diseases associated to nasal polyps

2.4.3.1. Samter’s triad

Samter’s triad or ASA triad is the syndrome of nasal polyposis, asthma and ASA intolerance, which was first described by Widal et al. in 1922. Samter and Beers (1967) clarified the relationship later. The most plausible basic mechanism underlying sensitivity to aspirin is

that inhibition of cyclooxygenase by aspirin and other cyclooxygenase inhibitors could divert the metabolism of arachidonic acid from the cyclooxygenase pathway to lipoxygenase pathway, thereby increasing the generation of leukotrienes (LTs) by removing prostaglandin E_2 (PGE_2) a dominant cyclooxygenase product of airways, which has an inhibitory effect on inflammatory cells and pathways (Szczeklik 1990). Patients tend to exhibit ”panmucosal” reactivity with reactive mucosa throughout the airway including the traceobronchial tree and sinuses. The sinus disease is frequently extensive, but can be managed with aggressive medical and surgical therapy. This triad has been found in 8-39% of patients with nasal polyps (Drake-Lee 1987, Maran and Lund 1990, Schapowal et al. 1995)

2.4.3.2. Cystic fibrosis

Cystic fibrosis (CF) is one of the most frequent hereditary diseases found in Caucasians and it is caused by an autosomal-recessive defect of the CFTR gene on chromosome 7 (coding for a chloride channel) (Kerem et al. 1989, Riordan et al. 1989, Rommens et al. 1989). To date, some 900 gene mutations causing CF has been identified (The Cystic Fibrosis Genetic Analysis Consortium, CFGAC: http://www.genet.sickkids.on.ca/cftr). The F508 mutation is the most common one (Estivill et al. 1997), but the frequency of mutations greatly varies between different populations. The frequency of F508 is found to be 65-80% in North American, British, Swiss and Dutch patients but only 51-58% in Spanish and Italian populations and 37% of black American patients (Deane and Schwartz 1997). The incidence of CF in Finland, 1:26500 new-borns, is one of the lowest in Caucasian populations (European Working Group for Cystic Fibrosis Genetics 1990) (Kere et al.1994). In Finland the incidence of CF is approximately one tenth that in other European countries, including the neighbouring countries of Sweden, Denmark, the former Soviet Union and Norway (Kere et al. 1989). The carrier incidence of CF varies in different populations. In Finland where CF is uncommon one in 80 is a carrier of the gene (Kere et al. 1989) but for example in the USA one in 25 Caucasians is a carrier of a gene mutation (Aitken and Fiel 1993).

The rarity of CF in Finland has been explained by genetic drift (Kere et al. 1989). The most common F508 mutation (Estivill et al. 1997) accounts for 45% of CF chromosomes and 394delTT mutation 30% of CF chromosomes in Finland (Kere et al. 1990, Kere et al. 1994). 394delTT is a specific Nordic mutation and this mutation is rare outside the Nordic countries (Kere et al.1994, Schwartz et al. 1994).

This defect causes abnormal transepithelial chloride transport across the respiratory and exocrine gland cell epithelium and impaired mucociliary clearance (Deane and Schwartz 1997). The typical clinical features for CF patients are an abnormally high concentration of chloride in the sweat, lung and paranasal infections, nasal polyposis, pancreatic insufficiency, male infertility and decreased female fertility (Aitken and Fiel 1993). However, the clinical expression of the states associated with the mutations is wide.

As many as 10% of children with CF may have concomitant nasal polyps (Maran and Lund 1990). Adult patients with CF have higher frequency of nasal polyps, reported to be 20-48% (di Sant' Agnese and Davis 1979, Aitken and Fiel 1993).

There are some reports in which the prevalence of CF mutations has been higher in infectious patients than in the average population. An abnormal distribution of CF heterozygotes was found in chronic bronchial hypersecretion (Dumur et al. 1990). Wang et al. (1998) found a higher frequency of CF mutation carriers among chronic sinusitis patients than in a normal control group. A recent study showed that in non-alcoholic pancreatitis the CF mutations are more common than in population on average (Sharer et al. 1998).

However, there are reports in which the CF frequency has not been increased. The prevalence of F508 mutation has been studied among 17 patients diagnosed with diffuse panbronchiolitis, but no mutation was found among them (Akai et al. 1992). In a study with severe nasal polyposis patients CF mutations were not detected to have an inactivation of the CFTR gene (Irving et al. 1997). The frequency was not increased among chronic bronchitis patients (Artlich et al. 1995).

2.4.3.3. Primary ciliary dyskinesia syndrome

The incidence of the autosomal recessive disorder, primary ciliary dyskinesia (PCD) syndrome, is estimated as being between 1 in 15000 to 30000 persons (Rott 1983). Patients with PCD syndrome are known to suffer from the presence of nasal secretions since the first day of life and this leads to recurrent upper respiratory tract infections with chronic sinusitis and nasal polyps up to 40% of them. Chronic and recurrent ear problems are found in up to 100% of the children (Pedersen and Mygind 1982). These are due to malfunction of the cilia, which is caused by a lack of dynein in microtubules sometimes even in 100% of cilia (Chao et al. 1982). The maximal manifestation of the illness, known as Kartagener's syndrome is characterised by chronic rhinosinusitis, chronic bronchitis with bronchiectasis and situs inversus, (Pedersen and Mygind 1982, Afzelius 1986). Male infertility is typical, but not 100% (Munro et al. 1994).

2.4.3.4. Churg-Strauss syndrome

Churg-Strauss syndrome is classified as a granulomatous vasculitis. It is characterised by the presence of a history of atopy, asthma, and eosinophilia in conjunction with a systemic necrotizing vasculitis. (Churg and Strauss 1951). In a study by Olsen et al. (1980) 69% (22/32) of Churg-Strauss syndrome patients had nasal manifestation and nasal polyps were seen in 11 of 32 patients (34%). Histopathologically, polyps removed from the nose show necrosis in association with eosinophilic exudate, severe fibrinoid collagen alteration, and granuloma formation with accumulation of epithelioid and giant cells that are called allergic granuloma (Mc Donald 1993).

2.4.3.5 Young's syndrome

This syndrome is characterised by recurrent respiratory diseases, azoospermia and nasal polyposis and it was first described from the north of England by Young in 1970 (Young 1970). The respiratory disease consists of severe chronic sinusitis, which may be associated with bronchiectasis. CF can be excluded because the patients have normal sweat chloride tests and pancreatic function. Their ciliary ultra

structure is normal (Frenkiel and Small 1991). The azoospermia in Young's syndrome is due to a blockage in the epididymis that is distinguishable from the defect in the vas deferens associated with CF. The patients have normal spermatogenesis. The prevalence of Young's syndrome is considerably higher than that of CF or Kartagener's syndrome. Only a few cases of Young's syndrome have been reported in the USA (Schanker et al. 1985, Hughes et al. 1987), but the incidence is remarkable higher in the United Kingdom (Hendry et al. 1993). It is responsible for 7.4% of cases of male infertility (Settipane 1996).

2.4.3.6. Nonallergic rhinitis with eosinophilia syndrome

Nonallergic rhinitis with eosinophilia syndrome (NARES) is a new entity and it was briefly described in 1979 by Jacobs, Freedman and Boswell. It was analysed in 1980 by Mullarkey, and in 1981 by Jacobs (Mullarkey et al. 1980, Jacobs et al. 1981). Patients with NARES have significant nasal eosinofilia but no evidence of allergic disease by history, physical examination, skin tests, or serum IgE levels. This syndrome is of special relevance because of the inflammatory functions of the eosinophils (Venge et al. 1987), the frequency of nasal polyps, possibly being a precursor of the triad of nasal polyposis, nonallergic asthma, and ASA intolerance (Samter and Beers 1968, Monoret-Vautrin et al. 1990). Twenty-nine percent of patients suffering from NARES had nasal polyps in a study by Monoret-Vautrin et al. (1990).

2.5. PATHOGENESIS OF NASAL POLYPS

The pathogenesis of nasal polyps is not clear. Many theories have been presented. Bernstein et al. found alterations of the bioelectricity of sodium and chloride channels in the nasal mucosa (Bernstein et al. 1995, Bernstein et al. 1997). They studied polyp and turbinate mucosa using histochemical and immunohistochemical methods. The samples were treated with protease to achieve disaggregation of the epithelial cells. Those cells were cultured on permeable collagen matrix supports. At the time of maximal transepithelial potential difference, the epithelial cells were mounted in modified Ussing chambers and exposed to a sodium-positive channel blocker (amiloride

hydrochloride) and to selected chloride-negative channel agonists (isoproterenol bitartrate and adenosine triphosphate). Epithelial cells obtained from the polyps exhibited higher transepithelial potential differences and equivalent short-circuit currents than turbinate cultures. The responses to amiloride, isoproterenol and adenosine triphosphate were also greater for polyp than for turbinate cultures. The following theory was proposed. Local release of inflammatory mediators could cause sodium absorption and chloride permeability to be higher in polyps than in turbinate epithelia. Increased sodium absorption may result in an increased movement of water into the cell and into the interstitial fluid. The resultant oedema may lead to growth and enlargement of the polyp (Bernstein et al. 1995).

Vasomotor imbalance is implied because the majority of patients with nasal polyps are not atopic and no obvious allergen can be found. Patients have often a prodromal period of rhinitis prior to occurrence of polyps (Drake-Lee 1987).

Formation of nasal polyps has been suggested to involve a rupture of the surface epithelium and the prolapse of the lamina propria to be the result of tissue pressure from an oedematous and infiltrated nasal mucosa (Tos and Mogensen 1977).

2.5.1. Chemical mediators

Numerous mediators have been found in nasal polyps. Cytokines, adhesion molecules and growth factors have been the main interest of research. Histamine, serotonin, LTs, norepinephrine, kinins, esterase, and possibly PGD_2 are found within polyp stroma (Settipane 1987) and also increased levels of IgA and IgE within nasal polyps (Chandra and Abrol 1974, Holopainen et al. 1979, Drake-Lee 1987). Rasp et al. (1999) found significantly increased levels of IgE in the nasal secretions of nonallergic patients suffering from nasal polyposis compared to controls. These levels were as high as IgE levels in the nasal secretion of allergic patients without nasal polyps. Histamine, in comparison to serum levels, has been found in 100- to 1000-fold concentrations within nasal polyps (Drake-Lee 1987). Inducible nitrid oxide synthesis expression is upregulated in nasal polyp disease and inducible nitrid oxide synthesis is localised in the polyp epithelial layer (Watkins et al. 1998).

2.5.1.1. Cytokines

2.5.1.1.1. Interleukins

Interleukins (ILs) are a family of mediators released by many inflammatory and non-inflammatory cells. In nasal polyps IL-1 is regularly found (Simon 1996) and it is found primarily in monocytes and to a lesser extent in polymorphonuclear cells (Liu et al. 1993). IL-2 may have involvement in nasal polyps because there are findings that nasal polyp mast cells are IL-2 receptor positive (Larocca et al. 1989). IL-3 has either not been found at all or only intermittently at low concentrations in some studies (Simon 1996, Bachert et al. 1997), but in other studies IL-3 has regularly been found in polyp stroma (Hamilos et al. 1995). IL-4 has been localised in eosinophils in nasal polyps (Nonaka et al. 1995). IL-5 plays a key role in eosinophilia and eosinophils are the only human leukocytes expressing receptors specific for IL-5 (Migita et al. 1991). IL-5 concentration in the nasal secretion of non-allergic patients suffering from nasal polyps reaches similar levels as in allergic patients without nasal polyps and these levels are significantly higher than those of controls (Rasp et al. 1999). IL-8 has been found in polyp mucosa but it is also found in normal nasal mucosa (Simon 1996). In vitro nasal epithelial cells, in some conditions can release IL-6 (Ohtoshi et al. 1991), but concentrations of IL-6 and IL-10 in patients suffering from nasal polyps did not differ from controls (Bachert et al. 1997). IL-13 messenger ribonucleic acid (mRNA) levels increase in nasal mucosa after a grass pollen allergen provocation (KleinJan et al. 1999).

2.5.1.1.2. RANTES

The concentration of a cytokine called RANTES (regulated upon activation, normal T-cell expressed and secreted) in polyp stroma has also been subject to contradictory findings. It has strong eosinophil chemotactic and activating effects (Rot et al. 1992, Alam et al. 1993). Some studies have regularly detected RANTES within polyp stroma (Beck et al. 1996, Teran et al. 1997), or even found increased levels of RANTES mRNA and protein (Hamilos et al. 1997, Allen et al. 1998), but others could not detect any differences with the controls (Bachert et al. 1997).

2.5.1.2. Adhesion molecules

Increased levels of VCAM-1 (vascular cell adhesion molecule 1) are found in nasal polyps (Beck et al. 1996, Hamilos et al. 1996). VCAM-1 is an endothelial surface molecule important for the adhesion of eosinophils to the endothelium (Jordana and Dolovich 1997).

2.5.1.3. Growth factors

2.5.1.3.1. IGF-1, TGF-

In recent years, several studies have drawn attention to growth factors in nasal polyps (Petruson et al. 1988, Ohno et al. 1992, Pyykkö et al. 1998). Petruson et al. (1988) found that nasal polyps from 15 patients expressed insulinlike growth factor I (IGF-I) immunoreactivity. Ohno et al. -specific mRNA in nasal polyps, as well as in the nasal mucosa from a patient with allergic rhinitis, but it has not been found in normal mucosa. d to play a central role in the pathogenesis of chronic inflammation and fibrosis. Basic fibroblast growth factor (bFGF) is a potent growth factor for angiogenesis, wound healing, thickening of epithelial membrane, fibrosis and epithelial and glandular hyperplasia (Pyykkö et al. 1998). In a study by Pyykkö et al. (1998) polyps and for comparison also saliva and rhinorrhea were assayed with commercially available ELISA (enzyme-linked immunosorbent assay) and all the nasal polyps showed high concentration of bFGF but human saliva only had small concentrations and it was barely detectable in patients with rhinorrhea.

2.5.1.3.2. Vascular endothelial growth factor

The gene code encoding vascular permeability/vascular endothelial growth factor (VPF/VEGF) and the genes encoding its receptors (kinase domain region (KDR) receptor and fms-like-tyrosine kinase -1 (flt-1) receptor) have been extensively analysed for angiogenesis and vascular permeability (Ferrara and Davis-Smyth 1997). On a molar basis, VPF/VEGF is known to be more potent than histamine in the induction of vascular hyperpermeability (Senger et al. 1983). Ito et al.

(1995) examined polyps using Northern blotting and found that all nasal polyps from four patients expressed VPF/VEGF and KDR mRNAs. Their in situ hybridisation experiments suggested that plasma cells are active in the synthesis of VPF/VEGF (Ito et al 1995). VPF/VEGF has also been found in mast cells in some studies but not from nasal polyps (Boesiger et al. 1998, Grützkau et al. 1998, Yamada et al. 1998). Thus, previous studies suggest that VPF/VEGF may be involved in the pathogenesis of nasal polyposis.

2.5.1.4. Cytology and cytological markers

2.5.1.4.1. Eosinophilia

Eosinophils are the main characteristic inflammatory cells of nasal polyps and they are found in 80-90% of all polyps (Drake-Lee 1987, Settipane 1996). Neutrophils predominate in polyps in patients with CF, PCD and Young's syndrome (Settipane 1996). The correlation between the degree of eosinophilia and the glucocorticoid response is linked to glucocorticoid receptors on the human eosinophils (Prin et al. 1989). On activation, eosinophils release inflammatory products from their granules, e.g. MBP, eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), LTs and platelet activating factor (PAF). When released, these products directly damage the epithelium of the upper and lower respiratory tract (Gleich et al. 1988, Harlin et al. 1988). Activated, degranulated eosinophils predominate in polyp stroma (Appenroth et al. 1998) and may contribute to the tissue damage resulting in polyp formation.

2.5.1.4.2. Mast cells

Mast cells are widely distributed in the connective tissues of mammals and other vertebrates, where they are frequently located in close proximity to blood vessels (Selye 1965, Galli et al. 1994). They have a well-established role in the immediate hypersensitivity reaction and chronic inflammation, but otherwise their functions are mysterious (Yamada et al. 1998). It has earlier been shown that mast cells occur about twice as frequently in nasal polyps as in the normal nasal respiratory mucosa (Cauna et al. 1972). Histamine has been localised in the cytoplasm of mast cells by histofluorescent techniques

(Bumstead et al. 1979). High concentrations of free histamine have been found in fluid extracted from nasal polyps (Drake-Lee and McLaughan 1982). Mast cells have been shown to secrete histamine, heparin (Azizkhan et al. 1980, Marks et al. 1986) and tryptase (Blair et al. 1997), too. Stimulated mast cells and cognate cultured cell lines produce and secrete a variety of cytokines including tumour necrosis factor (TNF) (Baumgartner et al. 1994). The mast cell granules of polyps are relatively small and they either lack the outer lamellar element or are largely degranulated (Cauna et al. 1972). The bFGF is localised to the secretory granules in mast cells and may be released through degranulation (Qu et al. 1998). In a study by Drake-Lee et al. (1984a) polyp mast cells showed varying degrees of degranulation with vacuoles predominating examined by transmission electron microscopy. Electron-lucent and electron-dense material was found in some granules; crystalline structures and scrolls, normally seen in cells prior to degranulation, were found in otherwise degranulated vacuoles. These findings were thought to support the theory that mast cell degranulation in nasal polyps is in some way different from the more rapid changes found in cells activated by the classical IgE-mediated pathway.

2.5.1.4.3. Other cells

The polyp stroma regularly contains neutrophils, lymphocytes, monocytes, plasma cells and macrophages, in addition to eosinophils and mast cells (Cauna et al. 1972).

2.6. TREATMENT OF NASAL POLYPS

For most patients the management of nasal polyps comprises a combination of medical and surgical therapies. The recurrence of nasal polyposis constitutes a serious clinical problem. Recurrence rates up to 29-53% have been reported (Blumstein and Tuft 1957, Jäntti-Alanko et al. 1985).

2.6.1. Medical treatment

The recurrence of polyps may be slowed down by long-term topical corticosteroid treatment. Steroids have a multifactorial effect initiated by their binding to the cytoplasmic glucocorticoid receptor cell. The number of glucocorticoid receptors is reduced by glucocorticoid treatment (Knutson et al. 1996), but studies have not indicated any development of tachyphylaxis to therapy in nasal polyposis (Pedersen et al. 1976, Lildholdt et al. 1997) or in any other airway disease. Virolainen and Puhakka (1980) found a recurrence rate of 46% in a patient group treated with intranasal steroid (beclomethasone diprionate) while the corresponding figure was 87% in the placebo group at one year after ethmoidectomy. In a double-blind parallel-group trial of budesonide versus placebo Holopainen et al. (1982) noted that the polyps diminished in number and size and total mean symptom scores and nasal peak flow values showed a statistically significant difference in favour of budenoside at the end of the four-month trial period.

Lildholdt et al. (1988) compared surgical removal of visible polyps with the efficacy of a single intramuscularly administered dose of betamethasone dipropionate and betamethasone disodium phosphate. In both groups, a nasal spray of beclomethasone dipropionate was used postinterventionally daily for one year. The results in terms of recurrences in the two treatment groups did not differ significantly.

With regard to therapy most authors primarily prefer conservative medical therapy with steroids, but because of the side effects of systemic steroids and the strong recurrence tendency of polyposis surgery is often necessary in cases, which involve severe nasal obstruction or secondary infections (van Camp and Clement 1994).

2.6.2. Surgical treatment

Intranasal polypectomy may be performed under local or general anaesthesia and is traditionally performed using a snare, which may be cutting or avulsing.

The first description of an intranasal ethmoidectomy based on careful anatomical studies was credited to Mosher in 1913. He concluded that theoretically the operation was easy but in practise it had proven to be one of the easiest operations, which can kill the patient (Mosher 1929). This was largely due to poor visualisation while entering the anterior cranial fossa during the surgery. Later, large series of patients mainly treated for polyposis in whom the overall complication rate was extremely low (Eichel 1972, Freedman and Kern 1979).

In transantral ethmoidectomy the ethmoids are approached via the Caldwell-Luc procedure, but this method is very rarely recommended. The Caldwell-Luc operation is primarily designed for chronic maxillary sinusitis but it has been utilised for the removal of generalised polypoid disease, for fungal sinusitis and for the removal of antrochoanal polyps.

Today removal of polyps and ethmoidectomy are done under endoscopic control. The anatomical knowledge derived from CT scanning has greatly enhanced precise removal of diseased tissue. If patients have recurrent sinusitis polypectomy and ethmoidectomy can be combined with uncinectomy and middle meatal antrostomy. The “shaver” is an ideal instrument to remove massive polyps. Mucosa and polyps are aspirated into a suction channel and cut by a blade oscillating in this channel.

2.6.2.1. Complications of surgical treatment

The most common complications of surgical treatment are bleeding and later postoperatively synechiae. Cerebrospinal fluid leak is a rare complication but patients with polyposis are at increased risk. Lacrimal and orbital injuries have also been reported (May et al. 1994). Mucocele which can be caused by inflammatory obstruction of the sinus ostia can also be secondary to surgical manipulation (Jacobson et al. 1982, Alsarraf et al. 1999).

3. AIMS OF THE PRESENT STUDY

In this study we wanted to find out the clinical course, outcome and diagnostic problems of nasal polyposis, and also to exam possible pathophysiologic and etiologic factors of two new research areas in nasal polyposis.

The purposes of the study were:

1. to elucidate the clinical course of nasal polyposis over a long period of time.
2. to examine the reliability of A-US in detecting fluid retention or mucosal thickening in patients with chronic polypoid sinusitis using sinus CT as the decisive diagnostic test.
3. to analyse the sense of smell in patients with long-standing nasal polyposis as compared to a healthy control material.
4. to investigate the occurrence of VPF/VEGF in nasal polyps of patients with chronic nasal polyposis for two decades as compared to normal nasal mucosa.
5. to find out the frequency of the two most common CF mutations in patients with chronic sinusitis with or without nasal polyps.

4. MATERIALS AND METHODS

4.1. PATIENTS AND CONTROL SUBJECTS

4.1.1. Patients

Studies I-IV are based on a series of 109 patients who underwent polypectomy alone or in connection with ethmoidectomy at the Department of Otorhinolaryngology, University Central Hospital, Helsinki in 1977-78. The patients were then divided into three groups according to the results of clinical and allergological investigations (Holopainen et al. 1979):

1. ASA intolerance = a definite history of ASA intolerance or a positive oral ASA provocation test (Juhlin et al. 1972).
2. AT = a history of atopy or positive nasal provocation results concordant with positive skin test.
3. INTR = diagnostic procedures for allergy revealed no specific etiologic factors.

Of the initial series of 109 patients, 85 attended a follow-up examination four years after surgery (Jäntti-Alanko et al. 1985). Now at 20 years after surgery, we did a new follow-up examination. From those 85 patients in the first follow-up six patients had died, 19 patients had moved away and two were not reached. Fifty-eight patients could be reached and were invited to the follow-up examination. Information on nasal symptoms, present and past history of nasal disorders, operations of the nose and paranasal sinuses, asthma-like symptoms, atopy, and ASA intolerance was gathered by a questionnaire sent to all subjects. Three of those 58 did not answer. Fourteen returned a completed questionnaire but did not come to the examination. Forty-one patients (26 men and 15 women, mean age 59 years, range 35-84 years) attended this study in 1997. These 41 patients were included in studies I and III. Study II consisted 40 of the 41 patients (25 men and 15 women), because A-US was not available at the time of one patient's control study. One maxillary sinus was "missing" because of a medial maxillectomy, leaving 79 sinuses for evaluation. Study IV included 39 of the 41 patients (24 were men and 15 women), because biopsies were not obtained from two patients.

Study V is based on 127 chronic sinusitis patients, 35 men and 92 female. The mean age was 44 years (range 7-83 years). The patients had been sent to the ENT (Ear, Nose and Throat) clinic because of chronic sinusitis. The criteria for chronic sinusitis were at least 8 weeks of persistent sinusitis symptoms and signs, or four or more sinusitis episodes per year (Lund and Kennedy 1995). On average the chronic sinusitis symptoms had started 12 years earlier, but the range was large from 1 to 40 years. Thirty-four percent (43/127) of the patients had nasal polyposis and 31% (40/127) had asthma. Ninety-one percent (116/127) of the patients had had one or more operations for the maxillary sinus cavity; either inferior meatal antrostomy (57 patients, 45%), endoscopic middle meatal sinus surgery with uncinectomia or middle meatal antrostomy (91 patients, 72%) and/or Caldwell-Luc (36 patients, 28%).

4.1.2. Control subjects

In study IV there were ten control patients who had no allergies anamnestically or nasal polyposis. The mean age of the control group was 44 years (range 17-77 years), 5 were men and 5 women. These control subjects underwent septoplasty (n=2), rhinoplasty (n=2), dacrocystorhinostomy (n=3), turbinoplasty (n=2) or decompression of the orbit (n=1) and during these operations a biopsy from the mucosa of the middle turbinate was taken with the permission of the patients. The diagnoses of these control patients were septal deviation (n=2), nasal deformation (n=2), dacryocystitis (n=3), hypertrophy of inferior turbinates and snoring (n=2) and endocrine exophthalmia (n=1).

In study III the olfactory thresholds were compared with the earlier published results of a study in which the age-adjusted reference interval of the olfactory threshold was estimated with the same method (Simola and Malmberg 1998). The control group in the earlier study consisted of 104 subjects (56 women and 48 men, aged 17–71 years, mean 39 years) with no acute or chronic nasal complaints.

4.2. METHODS

4.2.1. Long term follow-up study

The patients were re-examined and classified now as were earlier into three groups (ASA, AT, INTR). All the patients were examined as follows: physical examination, sinus CT, A-US, olfactory threshold measurement, odour detection test, skin prick tests, nasal smears, active anterior rhinomanometer, acoustic rhinometer and a biopsy from a polyp or from the mucosa of the middle turbinate, if no polyp was seen. Information on nasal symptoms, present and past history of nasal disorders, subjective estimation of sense of smell, operations of the nose and paranasal sinuses, asthma-like symptoms, atopy, and ASA intolerance was gathered by means of a questionnaire completed by all subjects.

4.2.1.1. Nasal polyposis in anterior rhinoscopy

The status findings in anterior rhinoscopy were classified into four groups: 0 = no polyps, 1 = polypoid mucosa, 2 = small polyps, 3 = one big polyp, 4 = large polyposis.

4.2.1.2. Imaging techniques

A-US examination of the maxillary sinus and sinus CT were done in the course of 2 hours. All 79 sinuses were examined with A-US by an experienced ENT resident (investigator 1) and a second A-US examination was performed on 70 of the sinuses by a radiologist (investigator 2). The two investigators had no knowledge of the patient's history, CT findings or the other investigator's results. The US findings recorded by investigator 1 were carefully analysed for reliability by comparison with CT evidence. The results obtained by investigator 2 were primarily used to assess the reproducibility of A-US diagnoses but they were also to find out the sensitivity and specificity of A-US in detecting fluid and mucosal thickening for two investigators.

4.2.1.2.1. A-US

The A-US equipment used was a Sinus 320 (Entomed AB, Sweden) with a printer connection. The probe diameter was 13 mm and the transducer frequency 3.5 MHz. The probe was applied to the anterior wall of the maxillary sinus beneath the opening of infraorbital nerve, with the patient leaning his head a little forward so that the line from the external auditory meatus to the lower margin of the orbit was horizontal (Revonta 1980). The maximal echo was achieved with small movements of the probe.

The A-US findings were classified into three groups: 1) almost normal mucosa = US echo received at a distance of $\leq$ 1.5 cm; 2) thickened mucosa = US echo from a depth of more than 1.5 cm but less than 3.5 cm; 3) total obliteration of the lumen or fluid level = US echo at a distance of $\geq$ 3.5 cm (= back wall echo). These criteria are modified from criteria by Revonta (1980). The results of US were compared to those of CT.

4.2.1.2.2. Sinus CT

CT scanning was done with a Somaton Plus 4 scanner (Siemens, Germany) and included coronal views to visualise all the paranasal sinuses and axial views of the maxillary sinus area. The coronal and axial images were done with a spiral technique (slice thickness 2 mm, a pitch of 2) and the images were reconstructed with a bone algorithm and viewed with a wide window (4000/900). The amount of fluid was estimated roughly in the coronal CT view by measuring the height of the sinus lumen and the height reached by the fluid level (expressed as percentage of sinus height ”volume”). The axial view of the maxillary sinus was also checked for the presence of fluid.

The thickness of the mucosal swelling was measured in the axial view in the area directly below the infraorbital nerve. The thickness was classified into three groups: 1) normal mucosa or mucosal thickening $<$ 0.5 cm, 2) mucosal thickening $\geq$ 0.5 cm, 3) total obliteration of the air space or fluid level.

Obliteration of the olfactory recesses was also evaluated from coronal

CT scans. The olfactory recesses are beneath the cribriform plates of the ethmoid bones, the superior turbinate being laterally and the nasal septum medially. They were recorded as open (totally/partly) or occluded (totally, both sides).

4.2.1.3. Sense of smell

The olfactory threshold was measured using a commercially available smell test kit (Olfacto-Labs, Berkeley, California). Olfactory detection thresholds were obtained using phenylethyl methyl ethyl carbinol sniff bottles. Subjects were asked to choose the bottle they thought contained the odorant, and the test was repeated three times at each step. The accepted olfactory threshold was the weakest concentration at which the subject picked the correct bottle all three times. The detailed method is described elsewhere (Amoore JE and Ollman BG 1983, Amoore JE and O' Neill RS 1986). The threshold was expressed in arbitrary logarithmic units (ALU), designated as "decismels" (dS). The concentrations in use were —25, —15, —5, 5, 15, 25, 35, 45, and 55 ALU. In the statistical analyses, the value of 65 ALU was used for those who could not identify the strongest concentration. The olfactory thresholds were compared with the earlier published results of a study, in which the age-adjusted reference interval of the olfactory threshold was estimated with the same method (Simola and Malmberg 1998). In brief, the age-related 95% reference interval was calculated by first fitting a linear regression model to the olfactory threshold data. Another linear regression model was fitted to the standard deviations of the residuals about the first regression. The reference interval was calculated as

$$f_1(\text{age}) \pm 1.96 \times f_2(\text{age})$$

where f_1 and f_2 are the functions of age fitted to the threshold data and the residuals, respectively. The threshold was considered as hyposmia, if it exceeded the upper age-related reference limit and 65 ALU as anosmia (Simola and Malmberg 1998) in study III. In study I the olfactory thresholds were compared with the reference values given by the manufacturer (—25 to 25 = normal sense of smell, 35 to 55 = hyposmia and over 55 ALU = anosmia).

The sense of smell of polyposis patients was also assessed using a detection test with 7 different odours. The odours used were coffee, cinnamon, spirit, vanillin, camphor, turpentine and gasoline. In this

test, detection also without identification of the odour was accepted. The patients were categorised into three groups; 1) patient detected all 7 odours, 2) patient detected 4 to 6 odours, 3) patient detected less than 4 odours**.**

4.2.1.4. Rhinomanometry and acoustic rhinometry

NR6-2 computerised active anterior rhinomanometer (G.M. Instruments Ltd., Glasgow, UK) was used to measure the nasal resistance of polyposis patients. The nasal resistance was measured on each side of a Broms 200 units circle and the total nasal resistance was calculated. The volume between the distances of 4 to 7 cm from the nostrils was measured with an A1/2 Acoustic Rhinometer (G.M. Instruments Ltd., Glasgow, UK). The sum of unilateral volumes was calculated for all patients. The nasal mucosa was decongested by inhalation of 0.5% xylometazoline hydrochloride nasal spray in each nasal cavity 15 minutes before the measurements. The values of nasal resistance and nasal volume were logarithmically transformed to achieve a fairly normal distribution, and these values were used in statistical analysis.

4.2.1.5. Allergy tests

Skin prick tests to 22 common environmental allergens (Soluprick® allergen solutions, ALK, Denmark) were performed on all patients. The patient was considered prick-test positive (atopic), if at least one allergen elicited a weal whose diameter was at least 3 mm larger than that of the negative control.

4.2.1.6. Nasal smears

The secretion eosinophils were scored as negative or positive according to a semiquantitative microscopic evaluation (Hansel 1951).

4.2.1.7. Biopsies

A biopsy was taken from a polyp or from the mucosa of the middle turbinate, if no polyp was seen. In control subjects the biopsy was taken from mucosa of the middle turbinate. The biopsy specimens were frozen in liquid nitrogen, sectioned and stained with hematoxylin and eosin for detection of structural components and inflammatory cells. The biopsy specimen was considered to show tissue eosinophilia if there were 20 or more eosinophils per 100 inflammatory cells (Holopainen et al. 1979).

4.2.1.8. Determination of VPF/VEGF expression

VPF/VEGF expression was determined using a mouse monoclonal antihuman VPF/VEGF antibody (MAB293, IgG2b class, R&D Research, MN, USA) raised against the 165 amino acid form of the polypeptide. The 5-µm frozen sections were rehydrated, incubated for 30 min in 0.3% hydrogen peroxidase in methanol at room temperature, and for 20 min in 5% normal horse serum at room temperature. The sections were incubated overnight at 4 °C with the primary antibody at a dilution of 1:24. A subsequent incubation for 30 min in biotinylated anti-mouse serum was followed by a 30 min incubation using reagents of the vectastain Elite ABC kit (Vector laboratories, Burlingame, CA, USA). Peroxidase activity was developed with 3-amino-9-ethyl carbazole (Sigma, St Louis MO, USA) for 10 min. Finally, the sections were stained with haematoxylin for 5 min. A pathologist (CHJW) who was blinded to the patient data examined all the samples.

The percentage of the surface epithelium that stained for VPF/VEGF was estimated and the intensity of staining was graded from 1 to 5. The percentage of the VPF/VEGF stained epithelium of nasal glands was also estimated, and the intensity was similarly graded. The amount of VPF/VEGF (x (unit) = a(%)*b/c(%)) was calculated using the equation: x = a (percentage of relevant epithelium) that stained for VPF/VEGF) * b (the intensity of staining) / c (percentage of relevant epithelium of all epithelium).

4.2.2. Determination of cystic fibrosis gene mutation carriers (394delTT and F508) in patients with chronic sinusitis

Genomic DNA was isolated from the whole blood specimens according to standard protocols. Positive controls from CF patients were used (Kere et al. 1994). The primers used were
5'-CTGGAGCCTTCAGAGGGTAAAAT-3' and
5'-CATGCTTTGATGACGCTTCTGTA-3' for F508, and
5'-CTTGGGTTAATCTCCTTGGA-3' and
5'-ATTCACCAGATTTCGTAGTC-3' for 394delTT.

Each reaction contained 10 µl 5 ng/µl DNA, 2 µl 2mM dNTP, 0.12 µl 5 U Taq polymerase (AB Applied Biosystems), Gold buffer polymerase (AB Applied Biosystems), 0.5 µl 20 µM of each primer, 0.2 µl $DMSO_4$ and 4.7 µl H_20. The reaction mixture was submitted to amplification for 30 cycles. The temperatures and times for 394delTT were denaturation 30 s at 95 ºC, annealing 40 s at 53 ºC and extension 40 s at 72 ºC. The temperatures for F508 were 1.5 min at 95 ºC, 1.5 min at 65 ºC and 1.5 min at 72 ºC, respectively. *Hinf*I digestion was performed for polymerase chain reaction (PCR) products to detect 394delTT mutations. The sizes of the amplified fragments were: 1) F508: for normals 148 and for carriers 145, and 2) 394delTT: after *Hinf*I digestion for normals 32 + 172 + 105 and for carriers 32 + 172 + 103 (GenBank AH006034). The PCR products were then electrophoresed on 6% polyacrylamide gels, silver stained and scanned.

4.2.3. Statistical analysis

The statistical significances between the ASA, AT and INTR groups in the occurrence of asthma and in the use of nasal steroids were tested by the Chi squared test and the degree of obliteration of sinuses in CT and the amount of reoperations were tested by the Kruskal–Wallis one-way analysis of variance (ANOVA) test (I).

To examine the sense of smell in polyposis patients, we compared their olfactory thresholds with the thresholds in the group of the

reference subjects (Simola and Malmberg 1998). Stepwise multiple linear regression analysis (STATISTICA for Windows, StatSoft, Inc., Tulsa, OK) was used to examine the associations between olfactory threshold and age, gender, polyposis in anterior rhinoscopy (0 = no polyps, 1 = polypoid mucosa, 2 = small polyps, 3 = one big polyp, 4 = large polyposis), nasal resistance, nasal volume, the degree of opacity of the ethmoidal sinuses, ASA intolerance, allergy, diagnosed asthma, and the number of polypectomies or ethmoidal operations in the patient group. The Kappa statistic was used to assess the agreement levels between the olfactory threshold and odour detection tests and between the subjective estimation of the sense of smell and the measurement of olfactory threshold and odour detection test. According to Altman (1994), kappa values of 0.20 or less reflect poor agreement, with values of 0.21–0.40, 0.41–0.60, 0.61–0.80 and 0.81–1.00 the strength of agreement is fair, moderate, good or very good, respectively. For these purposes, the ranges of the measurements were divided into three groups (olfactory threshold: —25 to 25, 35 to 55 and over 55 ALU; odour detection test: 7, 3 to 6 and less than 3 correct detections; subjective estimation: normal, impaired, loss of sense of smell). Comparison of groups was made with ANOVA and the Chi squared test. Spearman's rank order coefficients were calculated for the association between odour detection and olfactory threshold test of the sense of smell (III).

The statistical significances between the amount of VPF/VEGF and ASA intolerance, AT and IT groups were tested using the Kruskal–Wallis one-way ANOVA test. The Mann–Whitney U-test was used in comparisons of the amount of VPF/VEGF between the polyposis patients and the control patients. The Spearman correlation coefficient was used in comparisons of the amount of VPF/VEGF to the number of polyp operations, the degree of mucosal obliteration of ethmoidal sinuses in CT, the number of eosinophils, mast cells and the status findings (IV).

5. RESULTS

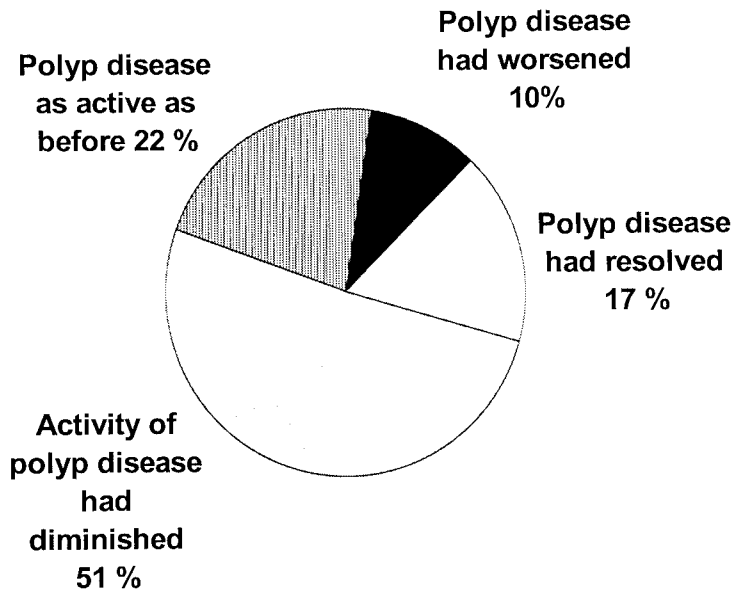
5.1. NASAL POLYPOSIS PATIENTS FOLLOWED FOR 20 YEARS (I-IV)

5.1.1. Symptoms (I)

Seventy-eight percent (32/41) of the patients suffered from continuous nasal symptoms and 69% (22/32) of these considered nasal blockage as their most annoying symptom. For some patients rhinorrhea (n=6) or sneezing (n=4) was the worst.

Seven of 41 patients stated that their polyp disease had resolved, 21 that the activity of the polyp disease had diminished, 9 that nasal polyposis was as active as before and 4 that their nasal polyposis had worsened (Fig. 1).

Fig. 1. Subjective estimation of the present activity of nasal polyposis in the 41 polyposis patients followed for 20 years after polypectomy or ethmoidectomy at the Department of Otorhinolaryngology, Helsinki University Central Hospital.



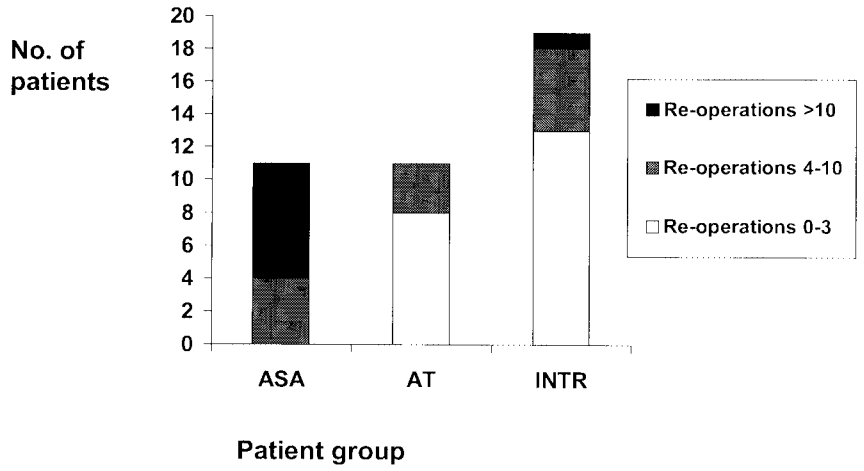
Fourteen of 58 patients sent a questionnaire completed by themselves but did not come to the examination. In this questionnaire 6 patients stated their polyp disease had resolved, 5 stated the activity of the polyp disease had diminished, one stated it was the same as twenty years ago and 2 stated that nasal polyposis had worsened. Altogether 71% (39/55) of the patients who completed questionnaires thought that their polyp disease had resolved or the activity of the polyp disease had diminished (unpublished result).

Bronchial asthma had been diagnosed in 44% (18/41) of the patients. Bronchial asthma was found in 100% (11/11) in the ASA intolerance group, in 36% (4/11) in the AT and in 16% (3/19) in the INTR group.

5.1.2. Operations during the follow-up period and present medical treatment (I)

Thirty-seven patients (90%) had had a re-operation during the previous 20 years. In those four non-operated patients rhinoscopy revealed many large polyps in two patients, polypoid mucosa in one patient and normal mucosa in one patient. Eight patients (20%) had had 11 or more nasal re-operations (re-polypectomy or anterior ethmoidectomy by endonasal approach), 7 (88%) of them belonging to the ASA intolerance group (Fig. 2). There was statistical significance in the amount of nasal re-operations in the ASA patients compared with AT and INTR patients ($P < 0.0001$). The Caldwell-Luc operation had been performed on 41% (17/41) of the patients, on both sides in 14 cases. Five patients had undergone the Caldwell-Luc operation before the initial study in 1977-8 but 2 of them had had re-Caldwell-Luc operations during the previous 20 years. Medial maxillectomy had been performed on one patient because of an inverted papilloma.

Fig. 2. Re-operations in 41 polyposis patients followed for 20 years after polypectomy or ethmoidectomy at the Department of Otorhinolaryngology, Helsinki University Central Hospital. Proportions of re-operated patients in the groups with acetylsalicylic acid (ASA) intolerance, atopy (AT) and intrinsic allergy-like disease (INTR).



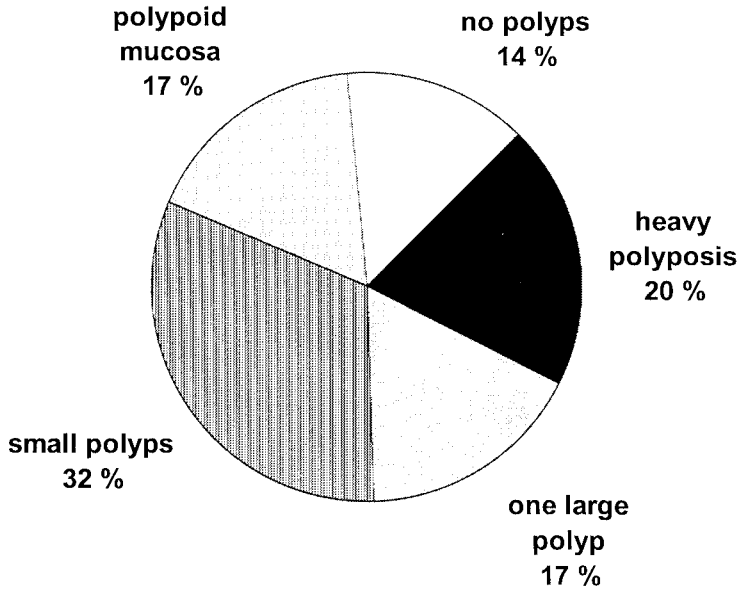
Patients had used many kinds of medications during the years but at the time of the follow-up examination topical corticosteroids were used by 49% (20/41) of all patients and by 91% (10/11) of ASA patients, 45% (5/11) of AT patients and 26% (5/19) of INTR patients. There was statistical significance in the use of topical steroids between ASA patients comparing with AT and INTR patients ($P < 0.003$). Thirty-seven percent (15/41) of the patients did not use any medication for nasal symptoms (one ASA intolerant, 5 AT and 9 INTR patients).

5.1.3. Status findings (I, II)

5.1.3.1. Nasal polyposis in anterior rhinoscopy

In anterior rhinoscopy polyps could be seen in 35 patients: heavy polyposis with many large polyps in 8, one large polyp in 7, some small polyps in 13 and polypoid mucosa in 7 patients. No polyps were seen in 6 patients (Fig. 3).

Fig. 3. Nasal polyposis in anterior rhinoscopy in 41 polyposis patients followed for 20 years after polypectomy or ethmoidectomy at the Department of Otorhinolaryngology, Helsinki University Central Hospital.



5.1.3.2. Nasal polyposis and sinus CT

According to the sinus CT all paranasal sinuses were obliterated heavily or moderately in 34% (14/41) of the patients and some of the sinuses in 39% (16/41) while only slight mucosal thickening was found in 27% (11/41) of the patients. Normal mucosa for all paranasal sinuses was not found. Mucosal changes were severest in the ASA intolerance group as compared to AT and INTR groups ($P < 0.0006$) (Fig. 4). The obliteration in ethmoidal sinuses was heavy in 46% (19/41), moderate in 32% (13/41) and slight in 22% (9/41) of the patients. Total obliteration in both olfactory recesses was found in 29% (12/41) of the patients (unpublished result). The operated maxillary sinuses were often markedly deformed or even destroyed (Fig. 5) and a "remodelling-phenomenon (Som et al. 1991) was seen in some ethmoid sinuses.

Fig. 4. Obliteration of paranasal sinuses in sinus CT in the 41 polyposis patients (divided to acetylsalicylic acid (ASA) intolerance, atopy (AT) and intrinsic allergy-like disease (INTR) groups) followed for 20 years after polypectomy or ethmoidectomy at the Department of Otorhinolaryngology, Helsinki University Central Hospital.

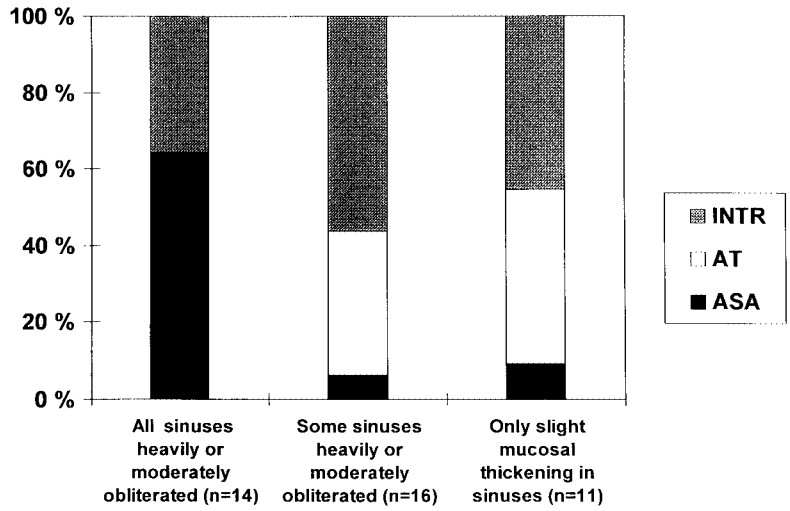
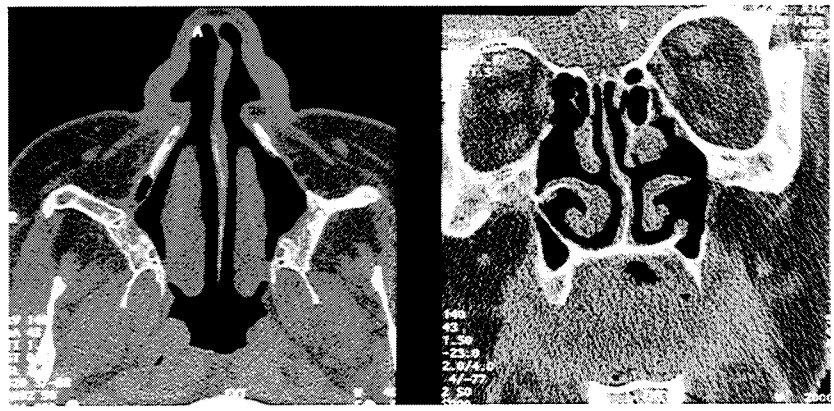


Fig. 5. The coronal CT image of a polyposis patient who has undergone two Caldwell-Luc operations 21 and 19 years earlier because of nasal polyposis and chronic rhinosinusitis. The maxillary sinuses are nearly destroyed.



5.1.4. A-mode ultrasound in the diagnosis of fluid retention or mucosal thickening in patients with chronic polypoid sinusitis (II)

Twenty of seventy-nine (25%) maxillary sinuses showed fluid levels or complete obliteration on the CT scans. Only 30% (6/20) of these abnormalities were detected with A-US. Fourteen fluid-containing or totally obliterated maxillary sinuses were not detected with A-US. According to the CT, fluid filled less than 30% of the lumen in 5 of these sinuses and in two an echo suggesting mucosal thickening was obtained. A-US findings were false positive in 14% (11/79) (Table I). In 6 cases, a back wall echo was received probably through a polypoid mass, although according to the CT, the maxillary sinus was not totally obliterated. For investigator 1, the sensitivity of A-US in detecting fluid or total obliteration was 30% and the specificity 81% and for investigator 2 28% and 69%, respectively.

The thickness of the mucosa was estimated in the 59 sinuses, which were not fluid-containing or completely obliterated. There was mucosal thickening in 30 (51%) maxillary sinuses according to CT. Twelve of these 30 (40%) were detected with A-US. For 15 sinuses (25%) A-US findings were false positive (Table II). In detecting mucosal thickening the sensitivity of A-US was 40% and the specificity 48% for investigator 1 and 50% and 58%, respectively, for investigator 2.

Table I. Comparison of A-mode ultrasound (A-US) results (Investigator 1) and computed tomography (CT) scanning interpretations in detecting fluid or total obliteration in maxillary sinus in chronic polypoid sinusitis patients (number of sinuses 79).
A-US f + = A-US echo indicative of fluid level or total obliteration of the air space
A-US f – = no indication of fluid level or total obliteration
CT f + = fluid level or total obliteration on CT sections
CT f – = no CT evidence of fluid level or total obliteration

		Sinus CT f	
Fluid level or total obliteration		+	–
A-US f	+	6	11
	–	14	48

Table II. Comparison of A-mode ultrasound (A-US) results (Investigator 1) and computed tomography (CT) scanning interpretations in detecting mucosal thickening in maxillary sinus without fluid or total obliteration of the air space in chronic polypoid sinusitis patients (number of sinuses 59).
A-US m + = A-US indicate of mucosal thickening
A-US m – = no A-US evidence of mucosal thickening
CT m + = mucosal thickening on CT scan
CT m – = no CT evidence of mucosal thickening

		Sinus CT m	
Mucosal thickness		+	–
A-US m	+	12	15
	–	18	14

The anterior wall of the maxillary sinus had been operated on in 30 cases. In these sinuses the sensitivity of A-US was 17% and the specificity 86% in detecting fluid or total obliteration, and 64% and 40% respectively, in detecting the mucosal swelling.

In the non-operated maxillary sinuses (n=49) the sensitivity of A-US was 36% and the specificity 77% in detecting fluid or total obliteration, and 19% and 53% in detecting the mucosal swelling.
The two investigators obtained identical A-US results in only 35 of 70 sinuses (50%).

5.1.5. Sense of smell (III)

5.1.5.1. Subjective estimation

Ten of 41 (24.4%) patients thought they had anosmia, 23/41 (56.1%) patients thought they had hyposmia and 8/41 (19.5%) thought they had a normal sense of smell.

5.1.5.2. The olfactory threshold

The average olfactory threshold was 39.9 ALU (SD 25.0) in the patient group and 13.8 ALU (SD 11.2) in the reference group. In 19 (46%) of 41 patients the threshold was greater than the age-related upper limit of the 95% reference interval (anosmia n=16, hyposmia n=3) (III). As compared with the 1.9% rate (2/104) in the reference subjects, the percentage was significantly higher (difference between the proportions 0.44, 95% confidence interval 0.29–0.60, $\chi^2 = 46.85$, $P < 0.001$). In the forward stepwise multiple linear regression analysis of all patients, the degree of obliteration of ethmoidal sinuses in CT ($P = 0.008$), polyposis seen in anterior rhinoscopy ($P = 0.023$), the logarithmically transformed total nasal resistance ($P = 0.01$) and gender ($P = 0.048$) (men had a better sense of smell than women) showed significant association with olfactory thresholds. The association of atopy and olfactory threshold was not significant ($P = 0.31$). In the ANOVA with degree of obliteration of ethmoidal sinuses and the logarithmically transformed total nasal resistance as covariates, the difference between men's and women's olfactory

thresholds was not significant ($F_{1,34}$ = 2.60, P = 0.12), the adjusted means being 36.8 ALU and 47.6 ALU, respectively. In the group of 29 patients with their olfactory recesses at least partially unobstructed, 5 (17%) had anosmia, 2 (7%) hyposmia and 22 (76%) normal olfactory thresholds, whereas, the corresponding figures were 11 (92%), 1 (8%) and 0 in the group with total obliteration of both olfactory recesses (χ^2 = 21.17, P < 0.0001) (unpublished result). In the ANOVA with age as a covariate, the difference between the patients with total obliteration of both olfactory recesses and the patients with at least partly open olfactory recesses was significant ($F_{1.38}$ = 26.71, P < 0.0001), the adjusted means being 64.2 and 29.3 ALU (unpublished result).

When the olfactory thresholds were compared with the reference olfactory threshold values given by the manufacturer anosmia or hyposmia was found in 61% (25/41) of the patients (I).

5.1.5.3. The smell identification test

Twelve of 41 patients detected less than 4 odours, 6 patients detected from 4 to 6 odours and 23 patients detected all 7 odours.

In the assessment of the agreement between the odour detection olfactory threshold and smell identification test, the value of kappa was calculated as 0.51 (95% confidence interval 0.28–0.73).

The Spearman rank order correlation coefficients between the subjective estimation of the sense of smell and the measurement of olfactory threshold and smell identification test were 0.62 (P < 0.0001) and 0.60 (P < 0.0001), respectively. The corresponding kappa values as measures of agreement were 0.38 (95% confidence interval 0.17–0.60) and 0.37 (0.16–0.58).

5.1.6. Allergy tests (I)

Fourteen patients had positive skin reactions to the allergen panel used. Eleven of them formed the AT group and 3 had also ASA intolerance and were taken into the ASA group.

5.1.6.1. Changes in the three groups (ASA intolerance, AT, INTR)

Eleven patients out of 41 belonged to the ASA intolerance group, 19 patients to the INTR group and 11 patients to the AT group. During 20 years the following changes had occurred: one ASA intolerant patient and 6 INTR patients were now skin prick test positive, and one of these INTR patients had also become ASA intolerant. Another INTR patient had become ASA intolerant, too. One ASA intolerant patient whose earlier skin tests had been positive and two patients classified as atopics were now negative on skin testing.

5.1.7. Tissue and secretion eosinophilia (I)

Biopsies were available from 40 patients and smears from all these patients. Tissue eosinophilia was found in 63% of the patients but secretion eosinophilia in only 25%. Six biopsies were from the mucosa of the middle turbinate and tissue eosinophilia was found in four of them. Twenty years earlier tissue eosinophils were positive in 94% and secretion eosinophils in 89%, respectively in 109 patients (Holopainen et al. 1979)

5.1.8. Vascular permeability factor/vascular endothelial growth factor in nasal polyps (IV)

VPF/VEGF was found on the surface epithelial cells of the mucosa, and in the nasal glands in both polyposis and control patients. VPF/VEGF was mostly found in the surface epithelium containing goblet cells. There was more VPF/VEGF in the surface epithelium in control patients than in polyposis patients, but it was not statistically significant (P = 0.0525). There were sections without any immunoreactivity for VPF/VEGF in the surface epithelium in 19/39 polyposis patients and in 2/10 control patients. The epithelium of nasal glands was found in 25/39 samples from polyposis patients and in 5 of these samples there was no immunoreactivity for VPF/VEGF, while immunoreactivity for VPF/VEGF was seen in the nasal glands of every control patient. There was more VPF/VEGF expression in the epithelium of nasal glands in control patients than in polyposis

patients ($P < 0.002$). VPF/VEGF immunoreactivity was also seen in stromal non-degranulated mast cells from two polyposis patients, however capillaries were not found in these biopsies. Almost all mast cells in the other samples both controls' and patients' were degranulated and in these cases there was no clear staining for VPF/VEGF.

There were no statistical significances between the amount of VPF/VEGF and asthma, ASA intolerance, AT or IT groups (the Kruskal–Wallis one-way ANOVA-test). The amount of VPF/VEGF correlated poorly to the number of polyp operations (Spearman index 0.19 in the surface epithelium and -0.19 in the epithelium of nasal glands). The correlation was also poor (0.32 and -0.07) when the amount of VPF/VEGF was compared to the degree of mucosal obliteration of ethmoidal sinuses in CT. There were no correlations either with the number of eosinophils, mast cells or with the status findings.

5.2. THE FREQUENCY OF CF MUTATIONS IN PATIENTS WITH CHRONIC SINUSITIS (V)

For this analysis there were DNA specimens from 127 patients. We found one carrier of the 394delTT mutation. This patient had no nasal polyps and her immunoglobulin levels were normal. However, her grandparents come from an area where the mutation 394delTT is enriched (Kere et al. 1994). None of the DNA specimens had a F508 mutation.

6. DISCUSSION

This study covers a period of 20 years of a chronic and probably life-lasting disease. The basic series consisted of 109 patients with nasal polyposis diagnosed in a clinical examination and the polyps were removed for microscopic examinations in polypectomy or ethmoidectomy operations in 1977-78 at the Department of Otorhinolaryngology, University Central Hospital, Helsinki. From the 85 patients in the first follow-up (Jäntti-Alanko et al. 1985) six patients had died, 19 patients had moved away from our area and two were not reached. Fifty-eight patients could be reached and were invited to the follow-up examination but 17 patients did not come. It is possible that the 41 who came could represent patients with the most troublesome symptoms. But 68% (28/41) of those who came and 79% (11/14) of those who only returned the completed questionnaire but did not come to the examination thought that their polyp disease had been resolved or the activity of the polyp disease had diminished and only 10% (4/41) and 14% (2/14) that their nasal polyposis had worsened and in that respect the patients in both groups were very much alike. Death or moving house are ”accepted reasons” not to come and so the selection was maybe not very markedly affected and this follow-up material could be rather representative of the original sample.

The patients were classified both in the earlier study in 1977-78 (Holopainen et al. 1979) and in this follow-up study 20 years later using rather similar criteria based on an allergy examination into three groups: ASA, AT and INTR. In our study INTR patients would have largely represented NARES patients but according to Monoret-Vautrin et al. (1990) this syndrome is a precursor of the Samter’s triad and in our study INTR patients had not become ASA intolerant with 2 exceptions over 20 years. The etiology of nasal polyposis is not known but the allergological division we used somehow subdivides the groups.

Besides the ”classical” allergologic criteria some more recent techniques were used in this analysis such as rhinomanometry,

acoustic rhinometry, olfactory threshold measurements, sinus CT and A-US.

Growth factors in nasal polyposis have been a subject of discussion in recent times. Of those the VEGF-family seemed theoretically interesting and it was much studied in our collaborators' laboratory. This was the reason we studied the occurrence of VEGF in nasal polyposis. Nasal polyps are found in 20-48% of CF adult patients (di Sant' Agnese and Davis 1979, Aitken and Fiel 1993). This is a hereditary disease caused by an autosomal-recessive defect of the CFTR gene on chromosome 7 (Kerem et al. 1989, Riordan et al. 1989, Rommens et al. 1989). There are some suspicions that the prevalence of carriers of CF gene in nasal polyposis and/or chronic sinusitis patients could be higher than in the population on average (Irving et al. 1997, Wang et al. 1998). Although this disease is very rare in Finland the possibility of overrepresentation of CF gene carriers in chronic sinusitis patients with or without polyps was studied in an independent study.

6.1 NASAL POLYPOSIS - CLINICAL COURSE DURING 20 YEARS (I)

Long-term follow-up studies of nasal polyps have earlier been reported by Blumstein and Tuft (1957), by Larsen and Tos (1994, 1997), and by Jäntti-Alanko et al. (1985) from this same material in our Department. The study published by Blumstein and Tuft (1957) consisted 77 patients with recurrent nasal polyps. This study claimed to show that allergy treatment prevented further recurrences in 47% of patients followed for 4.3 years. An additional 22% followed for 5 years partially benefited from treatment, as judged by the lengthened interval between polypectomies. Thirty-one percent of the patients followed from 5 to 7 years failed to respond to allergy treatment, either alone or combined with other forms of therapy. In this group there were many intractable asthmatics and poorest results were obtained in a group labelled ”nonsensitive”, those with negative skin reactions and no determinable specific etiology. This follow-up showed that patients with the highest recurrence of nasal polyps were ”nonsensitive” with a high incidence of asthma while patients with identifiable and controllable allergies had the lowest recurrence of polyps. In the present study AT patients seemed also to have a better prognosis, than

ASA and INTR patients although only 45% of AT patients used topical steroids.

Larsen and Tos described the clinical course of primary nasal polyposis in 180 patients in 1994, the follow-up time being 1 to 8 years (median 57 months). Ninety-six patients belonged to one or more of the etiopathogenetic subgroups of asthma, acute recurrent or chronic sinusitis, ASA intolerance or allergy. Sixty-six percent (118/180) did not undergo further polypectomies during the observation time (median 52 months) but they found that patients with asthma, acute recurrent or chronic sinusitis, ASA intolerance or allergy (39 of 62 patients belonged to one or more subgroups mentioned above) required more polypectomies and more topical steroid treatment than patients without these conditions. No significant difference in the number of polypectomies was observed between patients with asthma, sinusitis and allergy. The individual patient could be represented in one or more of the aetiopathogenic subgroups that were compared which was not the case in our study and so we can not compare these results unambiguously with the present study. The small number of patients with ASA intolerance did not allow statistical analysis in the study of Larsen and Tos (1994).

Larsen and Tos have also described another study of nasal polyp patients followed for approximately 8 years (38-145 months) after simple polypectomies. They found that ASA intolerant patients had the highest number of polypectomies, patients with asthma had more polypectomies than those without and the subjective estimation of olfaction representing anosmia or hyposmia was 45% of the patients. These results are very much like ours' except that in our study 80% of the subjects thought they had anosmia or hyposmia.

To the best of our knowledge studies with a follow-up time as long as 20 years with this group size (n=41) have not been published before.

It is a common clinical opinion that the recurrence and activity of nasal polyposis diminishes with time. Thus, it was surprising that nasal polyps were still affecting most of our patients. Nasal polyposis was seen in 85% of patients in anterior rhinoscopy. This percentage may be too low, because nasoendoscopy would probably have given even higher figures for polyposis (Penttilä et al. 1990). Also 68% of these 41 patients thought that their polyp disease was less disturbing or

had even disappeared over the 20 years, which then was not found to be the case. This means that the patients can not identify their polyp disease and they should be followed for years because relieving treatment can then be offered.

The prevalence of asthma (44%) in this study population was considerably higher than in population on average (Sears 1997). The differences in asthma prevalence were clear among these three groups but the groups were too small for analysis of statistical significance. The method used to collect data for the prevalence of asthma was anamnestic and the data was obtained using the questionnaire.

In most cases (16/18) bronchial asthma had been diagnosed before nasal polyposis but the first follow-up study (Jäntti-Alanko et al. 1985) already showed that bronchial asthma was diagnosed after nasal polyposis in 4 patients and now in this study 2 more patients had developed asthma after the first follow-up examination. The clinical relationship of nasal polyps to asthma has been analysed in many studies. It has been found that polyps developed earlier than asthma in 22% (Granström et al. 1992) to 33% of individuals (Schenk 1974) and the mean interval from onset of polyps to the development of asthma varied between 2 and 12 years (Schenk 1974). In Blumstein and Tuft's study (1957) they noted that patients belonging to the recurrent group are more likely to develop their asthma after polyps had been detected and repeatedly removed. There are also studies in which asthma developed first at a mean of 67% to 73% of the patients and the mean interval from onset of asthma to the development of polyps varied between 7 and 13 years (Schenk 1974, Larsen and Tos 1994)

It is probable that many patients had adjusted themselves to their polyp disease and its symptoms; nasal blockage, rhinorrhea, sneezing, sinusitis and hyposmia. Although 68% (28/41) of the patients thought that their polyp disease was less disturbing or had even disappeared during the 20 years anterior rhinoscopy showed that 85% of these patients still had nasal polyposis. Some patients (11/41) with massive polyposis in the anterior rhinoscopy or sinus CT were operated on and afterwards experienced considerable relief for at least nasal stuffiness.

There had been little intergroup change between ASA intolerance, AT and INTR groups over 20 years. One ASA intolerant patient and 6 INTR patients were now skin prick test positive and one ASA

intolerant patient for whom earlier skin tests had been positive and two patients classified earlier as atopics were now negative on skin testing. The new positive reactions can be considered as real positives, because skin reactivity decreases with increasing age (Simola et al. 1999). This decrease of skin reactivity may also explain why two patients classified as atopics were now negative on skin testing.

Tissue eosinophilia was found in 63% of the polyps but secretion eosinophilia in only 25%. Twenty years earlier tissue eosinophils in polyps were positive in 94% and secretion eosinophils in 89% of 109 patients (Holopainen et al. 1979). Both secretion eosinophilia and tissue eosinophilia were found most often in ASA patients who also had the most active nasal polyposis as judged by the degree of sinus involvement and number of re-operations and use of medication (91% used topical corticosteroids). Tissue eosinophilia seems to be an indicator of the active disease process in the nasal polyposis.

The patients with ASA intolerance had a higher recurrence frequency and a greater need for surgical treatment than the patients in the AT and INTR groups and in that respect they have a poor prognosis. This indicates that ASA intolerance is an important prognostic factor for the clinical course of nasal polyposis. AT patients seemed to have a better prognosis: they had fewer recurrences, milder mucosal pathology and they needed or used less intranasal steroids than patients in the ASA and INTR groups. Allergy is probably not a causative factor in nasal polyposis.

We need to know more about the pathophysiology of nasal polyposis to be able to develop more effective treatments and to identify more factors relevant to the prognosis of the disease.

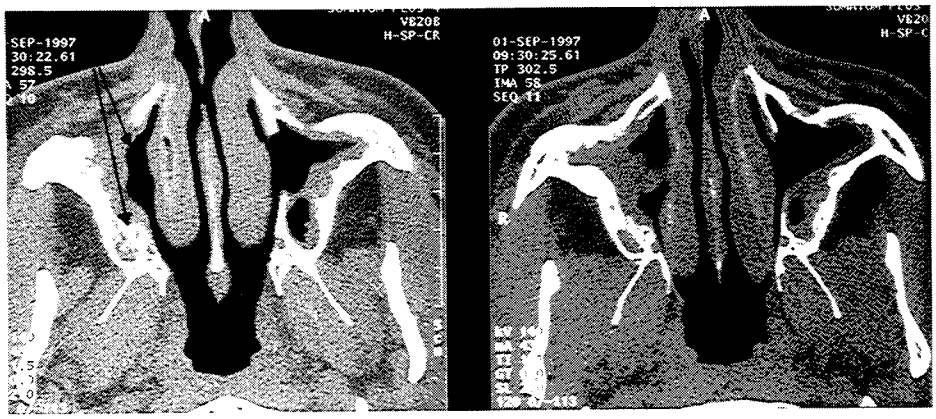
6.2. A-MODE ULTRASOUND IN THE DIAGNOSIS OF FLUID RETENTION IN PATIENTS WITH CHRONIC POLYPOID SINUSITIS (II)

Some earlier studies on the reliability of A-US in diagnosing sinusitis with fluid retention have produced rather contradictory results. This may partly depend on the patient material, which may have been very heterogeneous including both pure simple acute sinusitis patients and possibly more chronic or even operated cases. In this study, we

examined a very specific patient group to find out if A-US was useful in detecting fluid retention or in evaluating mucosal thickness of anterior wall in maxillary sinus in patients with chronic polypoid rhinosinusitis. The sinus disease of our patients was severe and marked pathologic changes in the antral mucosa were to be expected. In spite of this the poor correlation between CT and A-US in this study was surprising, as was the poor reproducibility of A-US results.

There could be many explanations for the discrepancy between the findings. The criteria of mucosal thickening are difficult to define. In the study by Revonta (1980) a healthy sinus with normal mucosa gave an echo from the depth of 1 cm or less, when the skin and soft tissues were tightly compressed between the probe and bone. In our study, we accepted an A-US echo from a depth of 1.5 cm or less as "almost normal", because many patients had thickened subcutis or deformed antral anterior wall due to earlier operations. The CT scans also showed that, maxillary sinuses were often totally deformed and the antral mucosal thickening was not evenly distributed. There were variations in thickness and polypoid changes within very small areas. This made it difficult to make an exact measurement on the CT scan and, very small alterations in probe positioning could cause large variations in the A-US echo (Fig. 6).

Fig. 6. Sinus CT (computed tomography) scan showing unevenly distributed polypoid masses in maxillary sinuses. In ultrasonography, very slight movements of the probe may produce two kinds of echoes in this area.



The rather large diameter (13 mm) of the probe may also have affected the results. A narrower probe could possibly be placed more accurately in position on these deformed anterior walls. The probe is narrower (8 mm, Sinuscan 102®, Finland) in the latest models of the A-US equipment.

Of course, the examination technique in use of A-US may also be questioned. We made the measurements in a rather small area corresponding to the area below the anticipated opening of the infraorbital nerve. Therefore, very small amounts of fluid were probably missed. But if an echo is received from a depth of less than 3.5 cm in the lower parts of the sinus, it is impossible to determine whether there is only mucosal thickening or a back wall echo from the smaller inferior recess of the antrum. A positive A-US finding suggesting the presence of fluid is regarded as an indication for sinus lavage. If such echoes are accepted as signs of fluid retention, many of the lavages will probable be negative. Then the benefit of using A-US is actually lost. The results of our study lead us not to recommend A-US to diagnose fluid retention or mucosal swelling in patients with chronic mucosal changes in their maxillary sinuses or if surgery has been performed on the anterior wall of the maxillary sinus.

6.3. SENSE OF SMELL IN CHRONIC NASAL POLYPOSIS (III)

According to our results, long-standing nasal polyposis was clearly related to an impaired sense of smell. In 19 (46%) of 41 patients the threshold was greater than the age-related upper limit of the 95% reference interval. As compared with the 1.9% rate (2/104) in the group of the reference subjects and with the 15% rate (16/105) in chronic rhinitis patients (Simola and Malmberg 1998), the percentage was very high. In a previous study of a group of chronic sinusitis patients (n=115) before functional endoscopic surgery, anosmia was found in 31% and hyposmia in 52% of the group (Delank and Stoll 1998).

Our patients had a greater residual olfaction in the odour detection test than in the olfactory threshold test for their sense of smell, but even though the patients in the first mentioned test could detect the odours, they could not identify them. This could be a sign of normal olfaction,

but also may be due to some kind of dysosmia or defective olfactory function (Douek et al. 1975).

In the assessment of the agreement between the olfactory threshold and the odour detection test, the value of kappa was calculated as 0.51 (95% confidence interval 0.28–0.73) and according to Altman (1994) in our data there seemed to be a moderate consistency between the simpler odour detection test and the more modern olfactory threshold.

Although anosmia or hyposmia were common, it seemed not to disturb the patients. One explanation might be that as most polyp tissue was found in the paranasal sinuses there were not so many polyps in the nasal cavities. Thus the patients may have felt symptom free because breathing via the nasal cavities was possible and they had grown accustomed to anosmia and head pressure over the years.

Total obliteration of both olfactory recesses and the degree of obliteration of the ethmoidal sinuses compared with olfactory threshold showed a significant correlation in our study. This is probably a typical conductive anosmia, where polyp masses obturate the upper airway passage. This is supported by the findings that postoperative steroid treatment or steroid treatment alone may improve the sense of smell by decongesting the mucosa (Jafek et al. 1987, Scott 1988). Many studies report the recovery of olfaction due to effective anti-inflammatory therapy (systemic or local steroids), surgical therapy or a combination of both, implicating conductive etiology (Jafek et al. 1987, Mott et al. 1997). Jafek et al. (1987) reported two anosmia patients with long-term reversal of the anosmia utilising a combination of surgical and pharmacological intervention. It would probably be optimal to combine surgery with steroid treatment every time to achieve permanent better results of olfactory function. But also irreversible impairment of the sense of smell has been found.

There were 5 patients who had anosmia although their olfactory recesses were open and one of these 5 patients had only slight obliteration in her ethmoidal sinuses in CT. This could be due to inflammatory degenerative changes of the nasal attic or ethmoidal mucosa, but also due to many polyp operations with scarring or also degenerative changes of the olfactory mucosa due to the chronic polyp disease and recurrent infections or their treatment causing sensory

olfactory destruction.

According to Simola and Malmberg (1998) a history of recurrent polyp operations was associated with poorer smell test results in a group of chronic rhinosinusitis patients. But only some of them had nasal polyps, so this group was more heterogeneous than ours. Hosemann et al. (1993) found that 87% of those patients in whom ethmoidal surgery did not improve the hyposmia or anosmia had already had earlier surgical interventions on their paranasal sinuses. However, in our study there was no significant association between olfactory threshold and the number of polypectomies or ethmoidal operations, this may be because all patients in our study had polyp disease but this was not true for all the patients in the study mentioned above.

Atopy and olfactory threshold did not show significant association ($P = 0.31$). In a study of Simola and Malmberg (1998) prick-test positivity was associated with better smell-test results in long-continuing rhinitis group. This may also be explained by the fact that all patients had polyp disease in our study this was not true for all the patients in the study mentioned above.

There was an inverse association between olfactory threshold and the logarithmically transformed total nasal resistance. Maybe there were some patients who had hyposmia not caused by nasal obstruction but instead by degenerative changes in the olfactory mucosa which caused sensoneural defect. For example in the present study there were two patients who had anosmia, but very good flow in rhinomanometry.

The male gender was associated with a better olfactory threshold in the regression analysis, but in the ANOVA with degree of obliteration of ethmoidal sinuses and the logarithmically transformed total nasal resistance as covariates, the difference between male and female olfactory thresholds was not significant ($P = 0.12$). There was no gender-association with the smell-test results for the group of the reference subjects (Simola and Malmberg 1998). Metabolic changes may affect the sense of smell, and in women e.g. hormonal changes may induce hypertrophic and hyperaemic effects in the respiratory mucosa of the nose as Douek (1975) has also speculated.

The method for smell identification and the kit used in this study for measuring olfactory threshold do not distinguish between a sensorineural and a conductive defect. A biopsy from the olfactory mucosa of the patients would have given further information about possible degenerative mucosal changes, which have been discovered in the olfactory mucosa of the patient with anosmia followed by influenza (Douek et al. 1975).

6.4. VASCULAR PERMEABILITY FACTOR/VASCULAR ENDOTHELIAL GROWTH FACTOR IN NASAL POLYPS (IV)

There are fewer blood vessels and they are of smaller diameter in nasal polyps than in healthy nasal mucosa (Cauna et al. 1972), but the permeability of capillaries and small vessels is high both in nasal polyps and normal mucosa, and the capillaries directly beneath the epithelium are mostly fenestrated (Cauna et al. 1972, Watanabe et al. 1980). Members of the vascular endothelial growth factor family (VEGF, placenta growth factor, VEGF-B, VEGF-C and VEGF-D) are currently known to be the major inducers of angiogenesis and lymphangiogenesis (Saaristo et al. 2000). It is known that VPF/VEGF is more powerful than histamine in increasing vascular permeability (Senger et al. 1983). The endothelia of small venules and capillaries become fenestrated within 10 minutes after VPF/VEGF application and VPF/VEGF increases capillary permeability in muscle and skin in rats and mice (Roberts and Palade 1995).

We used immunohistochemical staining in the determination of VPF/VEGF expression in our study. This method is sensitive but semiquantitative. Rather complicated calculations to estimate the degrees of positive staining were used. Northern analysis and mRNA isolation as used in later studies (Saaristo et al. 2000) would have given more exact results.

All chronic polyposis patients in our study had used corticosteroids to treat their disease, and it is known that corticosteroids reduce VPF/VEGF production (Ristimäki et al. 1998). VPF/VEGF could have played a more important role in active, untreated nasal polyposis.

Staining for VPF/VEGF were mostly found in the epithelium of the nasal glands. However, in two patients they were found in the mast

cells. Although polyps could be seen in anterior rhinoscopy on 33 patients, loose polypoid stroma was seen only in 25/39 biopsies and the remaining biopsies were probably not representative of the polyps. Capillaries and small blood vessels were not stained in the samples, but those in the pedicles of polyps, may probably have been more numerous and thus better suited for analysis.

VPF/VEGF expression was found in the surface epithelium goblet cells and in the epithelium of the nasal glands. This indicates the possibility that VPF/VEGF could take part in the mucus secretion from nasal glands and goblet cells. In a study done later than ours the expression of VEGF-C and its receptor VEGFR-3 was found in the developing and adult respiratory epithelium and in the nasal vascular plexus (Saaristo et al. 2000). In this study immunoperoxidase staining for VEGF-C occurred in the cytoplasm of the nasal respiratory epithelial cells as well as in the mucus-secreting glands and also weakly in the endothelial cells of the VEGFR-2- and VEGFR-3 positive vessels. It was suggested that secreted growth factor becomes concentrated on endothelial cell surfaces displaying its receptor and VEGF-C and VEGFR-3 could regulate the permeability of the vessels needed for the secretion of nasal mucus and regulation of the lumen of the nasal passages.

In another recent study (Coste et al. 2000) VEGF expression was evaluated in nasal polyps and control nasal mucosa and VEGF positivity was more frequent in inflammatory cells in nasal polyps (14/14) than in control nasal mucosa (3/6) ($P < 0.05$) and in the epithelium in nasal polyps (6/14) than in control nasal mucosa (2/6) (nonsignificant).

It has earlier been shown that mast cells occur about twice as frequently in nasal polyps as in the normal nasal respiratory mucosa (Cauna et al. 1972). The mast cell granules of polyps are relatively small and they either lack the outer lamellar element or are largely degranulated (Cauna et al. 1972). The biopsies in our study were mainly taken from the body or apex of the polyps. However, there are more mast cells in the pedicle of a polyp and mast cells are more degranulated in the pedicle region than in the other parts of a polyp (Sasaki 1986). In our study it would have been better to have taken the samples from the pedicle part of the polyp.

In our study mast cells were largely degranulated, except those mast cells which expressed VPF/VEGF in two patients. VPF/VEGF expression in mast cells has also been found in other studies. Recently, Boesiger et al. (1998) reported that bone marrow-derived cultured mouse mast cells and umbilical cord blood derived human mast cells produce and secrete VPF/VEGF. The release of VPF/VEGF could be regulated differentially from that of preformed mediators such as 5-hydroxytryptamine (5-HT) and histamine. Grützkau et al. (1998) reported that the HMC-1 human mast cell leukaemia cell line can constitutively express and secrete three isoforms of VPF/VEGF (of 121,165 and 189 amino acids). VPF/VEGF-positive mast cells have also been found more in the synovium of rheumatoid arthritis patients than in the synovium of non-rheumatoid arthritis patients by immunostaining (Yamada et al. 1998). The presence of VPF/VEGF in non-degranulated mast cells in two polyposis patients implies that the release of VPF/VEGF from mast cells in nasal mucosa could participate in the pathogenesis of polyps.

6.5. THE FREQUENCY OF CF MUTATIONS IN PATIENTS WITH CHRONIC SINUSITIS (V)

CF is associated with severe chronic infections in many organs. Especially respiratory tract infections, such as sinusitis, are common among CF patients and the prevalence of polyps have been reported to be 20-48% (di Sant' Agnese and Davis 1979, Aitken and Fiel 1993) in adult patients with CF. This has evoked interest to study whether CF heterozygotes are overexpressed in some chronic infections and nasal polyposis. Such an association has been found in chronic bronchial hypersecretion (Dumur et al. 1990), non-alcoholic pancreatitis (Sharer et al. 1998), and chronic sinusitis (Wang et al. 1998). There are also reports of diseases in which the CF carrier incidence has not increased: Akai et al. (1992) studied diffuse panbronchiolitis and Irving et al. (1997) severe nasal polyposis patients.

Interest in genetic diagnostics has increased in the past years, as the new technology has produced methods to displace genetic defects and thus to treat some hereditary diseases (Crystal et al. 1994, Wagner et al. 1998).

This study screening for two of the most common mutations in 127 chronic sinusitis patients identified only one carrier of a 394delTT mutation without nasal polyposis. The study population is somewhat small and thus we can not completely rule out some association between chronic sinusitis and CF. However, it is possible to find limits for the plausible values of prevalence. In a study with 127 participants, it can be calculated using binomial distribution that in order for the probability of finding none or at most one mutation to be at least 5% the prevalence of the mutation must be less than 0.024 or 0.036, respectively i.e. 5% level higher than 2.4% and 3.5%. The F508 and 394delTT mutations studied account for 75% of the CF mutations in Finland. It is quite improbable that those rare mutations that were not included in the study would be overexpressed in the chronic sinusitis population.

This study showed that in a population with a low-incidence of CF there was not an abnormal carrier distribution of CF gene mutations in a group of chronic sinusitis patients with or without nasal polyps. Routine screening of sinusitis patients with or without nasal polyps for CF mutations does not seem to have given any more information about the etiology of chronic sinusitis.

CONCLUSIONS

1. Sixty-eight percent of 41 patients thought that over 20 years the activity of their disease had decreased or the disease had been resolved. However polyps could be seen in anterior rhinoscopy in 85% of the patients, hyposmia or anosmia was found in 46% of the patients and in sinus CT all the paranasal sinuses were found to be heavily or moderately obliterated in 34% of the patients and some of the sinuses in 39% and normal mucosa through all the paranasal sinuses was not found suggesting an active disease and indications for some treatment and continuous follow-up. The patients with ASA intolerance had a higher recurrence frequency, and a greater need for surgical treatment than the patients in the AT and INTR groups.
2. A-US should not be used in patients with a history of chronic sinusitis, polyposis and especially in transantrally operated maxillary sinuses although it can be a valuable investigation method in cases of simple acute sinusitis. There are many sources of errors when A-US is used for diagnosing acute exacerbations of chronic polypoid or operated sinuses. Firstly, antral mucosal swelling with polypoid masses may be so thick as to give ultrasonographic echoes which are suggestive of fluid retention or total obliteration. Secondly, transantral operations on the maxillary sinus cause deformation of the anatomy, which makes diagnostic conclusions difficult.
3. Chronic nasal polyposis diminishes the sense of smell, probably mainly due to conductive mechanisms. But worsening of the sense of smell without simultaneous obstruction in the olfactory region as seen by CT in some cases may also be due to perceptive lesions.
4. The staining for VPF/VEGF in the mucosal surface and in the glandular epithelium of nasal polyps was weaker than in normal controls. In two patients strong staining for VPF/VEGF was found in the granules of mast cells, while the mast cells in other polyposis patients appeared to be largely degranulated. VPF/VEGF was not seen in the mast cells of control patients. This finding needs further study to find out if VPF/VEGF are secreted from mast cells and have a role in nasal polyp formation.
5. In the Finnish population with its low-incidence of CF there was

not an abnormal carrier distribution of CF gene mutations in the studied group of chronic sinusitis patients with or without nasal polyposis. Routine screening of sinusitis patients for CF mutations gives no more information on the etiology of chronic sinusitis.

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