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IMMUNODEFICIENCIES, PATHOGENS AND SEX IN UPPER RESPIRATORY DISEASES

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ACADEMIC DISSERTATION

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"Le microbe n'est rien,
le terrain est tout!"

Louis Pasteur,
sur son lit de mort *(1895)*

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1 ABSTRACT

Rhinosinusitis in adults and tonsillitis in children are among the most common respiratory ailments and cause significant morbidity and expenses. However, the predisposing immunological factors for these are largely unknown. The aim of this thesis was to study innate and adaptive immune disorders and properties of causative microbes in common upper respiratory diseases.

In the first two studies, different patient groups with adult rhinosinusitis (acute, severe chronic, and patients coming to sinonasal operations because of recurrent or chronic disease with or without polyposis) were studied. These groups were compared with each other and against control groups. Female patients were more prone to acute and recurrent rhinosinusitis as well as to other mucosal infections than male patients. Male patients, instead, had more sinonasal polyposis and chronic rhinosinusitis. Female patients with rhinosinusitis had more total and partial deficiencies of complement factor *C4B* genes than female controls or male patients. In concordance with this, increased serum levels of the C4 protein were seen in male patients scheduled for sinonasal operation. Complement system was upregulated in acute rhinosinusitis in female and male patients. Compared with controls, the levels of immunoglobulins IgG1 and IgG3 were lower and those of IgA and IgG2 higher in sinonasal operation patients.

In the third study, pediatric patients coming to tonsillectomy were studied. Children with various indications to operate were compared with each other and with age-matched unselected controls. There was an age-dependent difference in the prevalence of allergic diseases and sensitization to respiratory allergens between girls and boys. If a child had had recurrent infections earlier and had been operated for adenoidectomy, allergy and asthma were more common.

In the fourth study, group A streptococci from removed tonsillar tissue and blood from septicaemia patients were compared for their ability to bind the complement regulators C4BP and FH. It was found that there was no difference in the C4BP or FH binding ability between the virulent and less virulent strains suggesting that other factors may be more important for the streptococcal virulence.

As reported in our last study, rhinoviruses could also, for the first time, be detected from tonsillar tissue.

In summary, this study suggests that the development of sinonasal diseases is multifactorial. The underlying factors include predisposing immunological alterations that often are subtle in nature, distinct characteristics, like sex, of the patients as well as microbes that have adapted to living in upper respiratory mucosa and organs.

2 PUBLICATIONS

I Seppänen M, Suvilehto J, Lokki ML, Notkola IL, Järvinen A, Jarva H, Seppälä I, Tahkokallio O, Malmberg H, Meri S, Valtonen V. Immunoglobulins and complement factor C4 in chronic or recurrent adult rhinosinusitis. Clin Exp Immunol 145: 219-27, 2006

II Suvilehto J, Lokki ML, Notkola IL, Valtonen V, Meri S, Seppänen M. Low immunoglobulins, complement factor *C4* deficiencies and sex differences in rhinosinusitis and nasal polyposis. Submitted

III Suvilehto J, Seppänen M, Notkola IL, Antikainen M, Malmberg H, Meri S, Pitkäranta A. Association of allergy, asthma and IgE sensitization to adenoidectomy and infections in children. Rhinology 45: 286-291, 2007

IV Suvilehto J, Jarva H, Seppänen M, Siljander T, Vuopio-Varkila J, Meri S. Binding of complement regulators factor H and C4b binding protein to group A streptococcal strains isolated from tonsillar tissue and blood. Microbes Infect 10: 757-63, 2008

V Suvilehto J, Roivainen M, Seppänen M, Meri S, Hovi T, Carpén O, Pitkäranta A, Rhinovirus/enterovirus RNA in tonsillar tissue of children with tonsillar disease. J Clin Virol 35: 292-7, 2006

3 ABBREVIATIONS

ADCC	antibody-dependent cell-mediated cytotoxicity
AOM	acute otitis media
AP	alternative pathway of complement
APC	antigen-presenting cell
ARS	acute/intermittent (presumed bacterial) rhinosinusitis
ASA	aspirin (acetylsalicylic acid)
BCR	B-cell receptor
C	complement
C4BP	C4b-binding protein
CD	cluster of differentiation
CH50	complement classical pathway hemolytic activity
CP	classical pathway of complement
CRS	chronic rhinosinusitis
CRSsNP	chronic rhinosinusitis without nasal polyposis
CRSwNP	chronic rhinosinusitis with nasal polyposis
CT	computed tomography
CVID	common variable immunodeficiency
DAF	decay accelerating factor
EPOS	European Position paper on Rhinosinusitis and Nasal Polyps
FH	complement factor H
FcR	Fc receptor
GAS	group A streptococcus, *Streptococcus pyogenes*
GBS	group B streptococcus, *Streptococcus agalactiae*
GCS	group C streptococcus
GGS	group G streptococcus
HLA	human leukocyte antigen
HEV	human enterovirus
HRV	human rhinovirus
ICAM-1	intracellular adhesion molecule 1
IFNγ	interferon gamma
Ig	immunoglobulin
IL	interleukin
ISH	in-situ hybrization
iTreg	inducible T-regulatory lymphocyte
LP	lectin pathway of complement
LRT	lower respiratory tract
MAC	membrane attack complex
MBL	mannose binding lectin
MHC	major histocompatibility complex
NK	natural killer
NSAID	non-steroidal anti-inflammatory drug
NP	nasal polyposis
NPO	nasal polyposis only

NTHI	non-typeable *Haemophilus influenzae*
OME	otitis media with effusion
PAMP	pathogen-associated molecular pattern
PRR	pattern recognition receptor
PCR	polymerase chain reaction
R	rhinosinusitis
RNA	ribonucleic acid
RT-PCR	real-time polymerase chain reaction
RRS	recurrent rhinosinusitis
sIgA	secretory IgA
sCRS	severe chronic rhinosinusitis
SNO	sinonasal operation
Th	T-helper lymphocyte
TLR	Toll-like receptor
TNF	tumor necrosis factor
URT	upper respiratory tract
URTI	upper respiratory tract infection

4 INTRODUCTION

Upper respiratory tract (URT) diseases are among the most common ailments in humans. Little is known yet about possible immunological predisposing factors. URT diseases commonly involve inflammation and are sometimes caused by infectious agents. For reasons that only recently have begun to unravel some individuals have an increased susceptibility to these diseases.

All living plants and animals have their own ways to recognize and react to harmless and harmful organisms and substances. These mechanisms can be unique or shared by other species, and they can be even used by intruders to inflict damage to the host. Vertebrates have developed a sophisticated immune defense system to protect themselves from microbial attack.

Innate immunity provides a strong barrier to protect us from attacks by pathogens. However, only a limited number of deficiencies of innate immunity have been found to make an individual vulnerable to infections. Complement is a very powerful effector mechanism of both innate and adaptive immunity. Totally defective complement pathway function poses a serious risk for infections. Due to its three-pathway organization partial defects of one pathway can be, to an extent, compensated by the remaining other pathways.

C4 is a key protein of the classical and lectin pathways. Partial deficiencies in this most polymorphic protein of the complement system are common. Preliminary data suggest that even partial deficiencies of C4, together with immunoglobulin deficiencies, may predispose to severe rhinosinusitis. However, it is not known how partial deficiencies of C4 or immunoglobulins associate with less severe forms of rhinosinusitis.

In vertebrates, females are often more immunocompetent than males. Females and males have varied susceptibility to several infections and autoimmune diseases. These differences are not solely explained by anatomical differences or by rare X-linked primary immunodeficiencies found exclusively in males. An individual's sex is thus the most easily recognized phenotypic factor that may associate with intrinsic differences in immune responses. In clinical otorhinolaryngological practice, for unknown reasons, a relative excess of females coming to sinonasal operations is commonly noted.

In sinonasal disease refractory to anti-inflammatory and/or antimicrobial treatment, the only remaining therapeutic option is surgery. Unfortunately surgery does not always provide satisfactory results. If

the immunological background remains unrecognized we cannot direct treatment to the cause of the problem. Discovery of new risk factors for rhinosinusitis could potentially lead to the development of new forms of therapy and further help in the avoidance of unnecessary surgery.

Tonsillar tissues form the ring of lymphoid organs surrounding the upper respiratory and alimentary tract (Waldeyer's ring). This mucosal lymphoid tissue takes part in presenting foreign antigens to the adaptive immunity. Despite the fact that operative removal of the adenoid or tonsils are among the most common operations to pediatric patients, many open questions remain. There are contradictory reports about adenoidectomy as a predisposing factor for allergy later in life. Rhinovirus, one of the most common agents causing pharyngitis and common cold, has been recognized as an important precipitator of asthma exacerbations. It has been detected in all other parts of the respiratory tract, but not in tonsils. Group A streptococci are considered to be the most important pathogens in acute tonsillitis but they are also found in healthy tonsils. Is the reason for this survival an ability to escape local immune attack, including that by the complement system?

The purpose of this study was to explore selected immunological features of the host and properties of pathogens and study whether they affect the susceptibility to common URT diseases leading to operations.

5 REVIEW OF THE LITERATURE

5.1 Immunity in the upper respiratory tract

Mucosal immunity in sinonasal diseases is incompletely understood (Ramanathan et al. 2007). In chronic rhinosinusitis (CRS) the effectors and mediators of adaptive immunity have been studied widely, but knowledge concerning innate immunity is still very limited. We know that innate immunity mechanisms are responsible for most of pathogen recognition, destruction and disposal. Only a limited amount of pathogens are capable of penetrating the multiple barriers of innate immunity. Sometimes, for example in the tonsillar tissue, this may be allowed for the purpose of sampling material to stimulate adaptive immunity responses (Nave et al. 2001).

Differences between innate and adaptive responses are shown in **Figure 1a** and clinically relevant defects in these responses in **Figure 1b** (Fokkens et al. 2000). The humoral arm of innate immunity is capable of acting immediately against microbes with potent antimicrobial substances. It also triggers the cellular arm of innate immunity for action by opsonization of microbes and recruiting phagocytic cells to remove the foreign material. Pattern recognition receptors (PRRs) can recognize pathogen-associated molecular patterns (PAMPs) and also non-viable host structures (Ramanathan et al. 2007). These receptors (toll-like receptors, TLR; mannose receptor, ManR; scavenger-receptor, ScaR; CD14, CD36 and Marco) on the surfaces of antigen presenting cells (APCs) are capable of capturing and processing microbial structures and inducing inflammation and immune reactions. It is important that both exogenous and endogenous, potentially harmful, material is rapidly neutralized and then removed from the system with effective phagocytosis to allow inflammation to attenuate (Meri 2007). Different pathogen recognition mechanisms of innate immunity can trigger and upregulate adaptive immune responses that links these two arms of defense.

B-lymphocytes are able to recognize microbial polysaccharides directly with their surface receptor (B-cell receptor, BCR), but recognition of peptides requires T-cell help (Bondada et al. 2005). Antigen presenting cells (dendritic cells, macrophages, B-cells) take in microbial and non-viable host protein structures with their specific receptors and process them into peptides that can be presented to naive CD4+ T-lymphocytes on class II major histocompatibility complex (MHC II). Presentation occurs in the lamina propria of mucosal surfaces or in the local lymphoid structures, in the upper respiratory tract in the Waldeyer's (tonsillar) ring. These lymphocytes can be primed to T helper one (Th1), T helper two (Th2), inducible regulatory Tcells (iTreg) or T helper 17 (Th17)-

lymphocytes depending on the nature of stimuli and local cytokine environment. T-lymphocytes induce the proliferation of antigen-specific B-lymphocytes and production of immunoglobulins (Igs) by plasma cells that can then undergo clonal multiplication (Janeway 2005; Martinez et al. 2008).

Immunoglobulins, the main humoral effectors of adaptive immunity have an interesting dual role on innate immunity. They activate the classical pathway of complement to induce lysis of a recognized microbe. They are also able to bind and promote elimination of both large fragments C3b and C4b and complement-derived anaphylatoxins C3a and C5a and limit potentially harmful inflammation. In this way adaptive immunity can regulate the activity of the innate immunity (Basta 2008).

Figure 1a. The different sections and effector mechanisms of immunity.

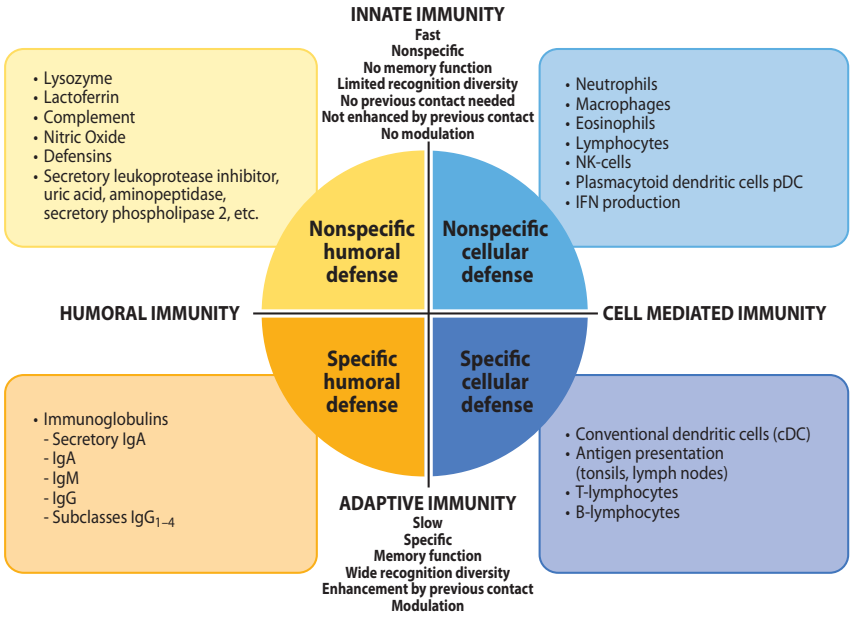
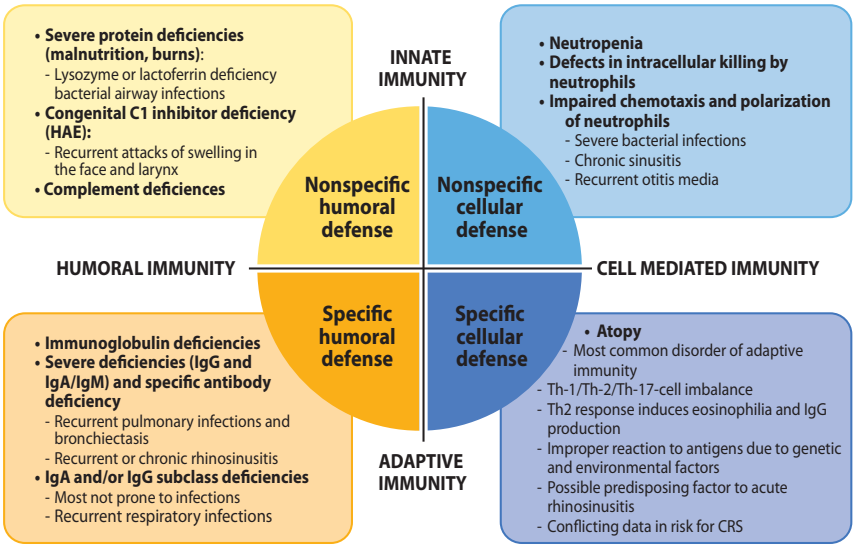


Figure 1b. Clinically relevant defects in different sections of immunity.



5.2 Innate immunity

In upper airways, the mechanisms of innate immunity provide us the primary defense against pathogens. Several independent and overlapping innate mechanisms make their evasion difficult. Nonspecific and microbe-specific innate immune defense mechanisms in the sinonasal cavity and clinically relevant defects in these systems associated with rhinosinusitis are listed in **Table 1** (Fokkens et al. 2000). Even in the case of passing this first barrier, information on the identity and the nature of the pathogen is passed on to the adaptive immune system for secondary response.

5.2.1. Cellular innate immunity

The cellular part of innate immunity consists of several cell types. Neutrophils can phagocytose and destroy opsonized pathogens, especially extracellular ones. Monocytes, which develop into macrophages, are more efficient against intracellular pathogens. Macrophages are important both in phagocytosing cells and nonviable or foreign material and in directing immune responses by the production of cytokines. They also act as APCs for adaptive cellular immunity (Godthelp et al. 1996). Eosinophilic cells act against large pathogens such as parasites and release enzymes and proteins to perforate cell membranes. They are also effector cells in allergic inflammation (Prussin et al. 2006). A special type of lymphocytes, natural killer cells (NK-cells) can kill pathogens and infected host cells with secreted substances and also produce cytokines (interferon-gamma, IFNγ) that enhance adaptive immunity (Janeway 2005).

Table 1. Upper airway innate defense mechanisms and their defects in chronic rhinosinusitis.

FACTOR	MECHANISMS	EFFECT	DEFECT IN CRS
BARRIERS			
Ciliary function	Mucociliary transport Increased flow in infection and due to airborne irritants	Eliminating pathogens by transport to alimentary tract	Trauma (surgery etc.) Viral infection Radiotherapy Primary ciliary dyskinesia (PCD) Toxins, air pollution, medications
Mucosal epithelial cells	Permeability to plasma PAMP recognition	Physical barrier Pathogen recognition	Trauma Viral infection Radiotherapy
Mucus	High molecular weight glycoproteins Salt consentration	Physical barrier protecting epithelial cells Antimicrobial factors suppress pathogen growth	Cystic fibrosis
SECRETED NONSPECIFIC ANTIMICROBIALS			
Lysozyme	Destroys peptidoglycan cell wall of bacteria	Toxic to fungi Needs lactoferrin, antibody-complement complexes or ascorbic acid against Gram-negative bacteria	
Lactoferrin	Binds iron Protects from hydroxyl radicals	Effective against Candida	
Others	ß-defensins, secretory leukocyte protease inhibitor , secreted phospholipase A2, cathelicidins, nitric oxide	Inhibit microbial growth Direct microbicidal activity Recruit phagocytic cells Develop adaptive response	ß-defensin2 decreased in epithelial cells in chronic rhinosinusitis with nasal polyposis (CRSwNP) (Claeys et al. 2005)
ACUTE PHASE PROTEINS			
Complement	Pathogen opsonization Phagocyte activation Lysis of bacterial cells	Enhances phagocytosis Direct killing of pathogens	
Serum amyloid A	Opsonization	Bind directly to Gram+ bacteria	
Surfactant proteins SP-A and SP-D	Bind and agglutinate non-self structures (bacteria, fungi, allergens, environmental inorganic substances)	Initiate and enhance immune cell ingestions and killing of targets	Decreased SP-A levels in CRSwNP (Ramanathan et al. 2007)
SPECIFIC RECEPTORS			
Pattern recognizing receptors (PRRs)	Recognize pathogen associated molecular patterns (PAMPs) Opsonization Signaling molecules	Promote phagocytosis	
Toll like Receptors (TLR1-9)	Signalling in macrophages, dendritic cells, endothelial cells and epithelial cells	Induce local primary immune defensive mechanisms Alert adaptive immune system Induce immune tolerance to normal flora?	TLR9 decreased in CRSwNP (Lane et al. 2006)

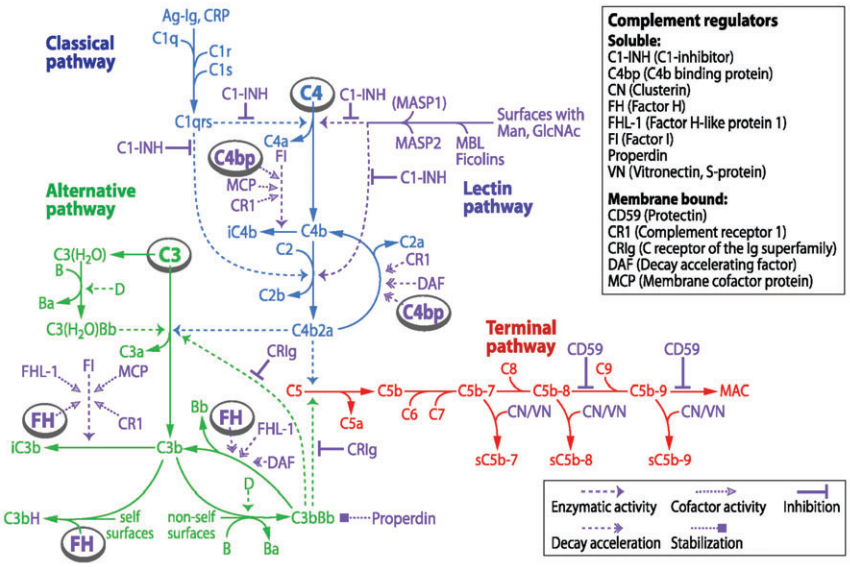
5.2.2 Complement activation and regulation

Complement (C) proteins form the major effector mechanism of innate humoral immunity. The C system consists of about 35 components forming a three-pathway system, where proteins are activated in a cascade fashion with potent amplification steps and effective regulatory factors (**Figure 2**). The three pathways enable a very large repertoire of triggers and functions for C. Primary roles of C proteins are to activate the inflammatory response, opsonize microbial pathogens and nonviable host components (cellular debris, apoptotic cells etc) for killing and phagocytosis and to lyse susceptible organisms. Complement is also a very important link between innate and adaptive responses through receptors in B-lymphocytes and APCs. Complement activation is normally targeted and very tightly regulated because its powerful destructive capacity may also damage the host.

Through a complex series of protease-activated cleavages of C proteins and their interactions the three pathways unite for a terminal pathway (**Figure 2**). The resulting membrane attack complex (MAC) is capable of forming transmembrane pores to promote complement-mediated lysis of cells.

Complement activation is tightly regulated by membrane bound and soluble regulators at three main levels (**Figure 2**). First, the initiation step of the classical pathway is regulated by C1-esterase inhibitor (C1-INH), which is a serine proteinase inhibitor (serpin) (Du Clos 2008). Secondly, the C3 and C5 convertases are regulated by C3b inactivator (factor I), soluble regulatory proteins C4b binding protein (C4BP) and factor H (FH) and by the membrane bound regulatory proteins CD55 (decay accelerating factor, DAF), CD46 (membrane cofactor protein, MCP) and CD35 (complement receptor 1, CR1) (Hourcade et al. 1989; Giannakis et al. 2003). The newest membrane regulatory proteins are C2 receptor inhibitor trispanning (CRIT), complement receptor of the immunoglobulin superfamily (CRIg) and the CUB and sushi multiple domains protein (CSMD1) (Inal et al. 2002; Helmy et al. 2006; Kraus et al. 2006). Properdin stabilizes the alternative pathway C3 and C5 convertases increasing their activity (Liszewski et al. 1996). Thirdly, the MAC formation is controlled by soluble MAC inhibitors S-protein and clusterin and by the membrane bound inhibitor CD59 (protectin) (Davies et al. 1993; Tschopp et al. 1994; Schvartz et al. 1999).

Figure 2. The complement system. The parts included in this study encircled and bolded.



Special thanks to Matti Laine for comprehensive illustration. (Modified from Matti Laine)

5.2.3. Complement function and deficiencies

The classical (CP) pathway of complement is activated by IgM- or IgG-containing immune complexes, by some PRR molecules (CRP and serum amyloid P-ligand complexes) and phospholipids in ischemic and apoptotic cells (**Figure 2**). The lectin pathway (LP) uses CP components, but is activated by mannose binding lectin (MBL) and by ficolins recognizing repeating simple carbohydrate patterns in microorganisms, apoptotic cells and occasionally glycosylated IgA or IgM bound to antigens. The alternative pathway (AP) can be activated by bacterial components (lipopolysaccharide (LPS), teichoic acids), fungal cell wall polysaccharides, virus-infected cells (measles, influenza, Epstein-Barr-virus), IgA containing immune complexes, C3 nephritic factor (C3NeF), cobra venom factor, some tumor cell lines and deoxygenated sickle cells. The AP can also be initiated through CP activation. It is also capable of autoactivation in the absence of inhibitory signals. A special feature of the AP is amplification of its own activation to further enhance opsonization and to promote activation of the terminal pathway.

In host defense, C-dependent opsonization is most important against infections by encapsulated extracellular bacteria like *Hemophilus influenzae* and *Streptococcus pneumoniae*. Increased susceptibility to infections caused by these bacteria is seen in individuals with deficient antibody production, neutrophil function or lack of C3. MBL variants are also associated with pyogenic infections in young children (Turner 1998). Gram-negative bacteria are susceptible to complement-dependent lysis. Individuals with deficiency of C3, any of the MAC components or properdin have an increased incidence of disseminated neisserial infections, notably of meningococcal meningitis (Densen 1991).

Complement promotes inflammation by anaphylatoxins C5a and C3a, which are cleaved from C5 and C3 during complement activation. These act also as chemotactic factors for neutrophils and macrophages. C5a also prevents apoptosis of neutrophils by prolonging their survival and promoting accumulation to inflammation site. They also affect the T-cell responses to antigen through their effects on lymphocytes and APC activity.

Damaged tissue compounds and apoptotic cells are recognized by multiple receptors and opsonins and can activate complement through several pathways. Timely clearance of nonviable material is needed to avoid the development of autoimmunity. Failure of complement-dependent opsonization due to early CP deficiencies (C1, C4 and C2) can lead to accumulation of apoptotic cells and persistence of autoantigens, characteristic of systemic lupus erythematosus (SLE) (Meri 2007). Factor D and properdin deficiencies result in an inability to activate the AP and to an increased susceptibility to neisserial infections. FH and FI deficiencies and the presence of C3NeF can lead to a severe acquired C3 deficiency and lack of regulation of fluid phase C3 convertases. As a consequence infections and membranoproliferative glomerulonephritis type II may develop. Serum carboxypeptidase N deficiency leads to a failure to control C3a, C5a and bradykinin and results in recurrent angioedema and urticaria. Recurrent angioedema (HAE) is a result of loss of regulation of C1s, C1r and kallikrein due to C1-INH deficiency (Nzeako et al. 2001). FH, FI and CD46 mutations can result in a lack of regulation of membrane C3 convertases and atypical hemolytic uremic syndrome (aHUS). Furthermore, polymorphisms in FH predispose to age-related macular degeneration (AMD) (Edwards et al. 2005; Haines et al. 2005; Klein et al. 2005). Lack of DAF and CD59 leads to a failure to regulate complement activation on autologous blood cells and results in paroxysmal nocturnal hemoglobinuria (PNH) (Meri 2007).

5.2.4 Complement evasion by pathogens

Innate immunity forms the major barrier protecting the host from potentially harmful exogenous and endogenous factors. Survival strategies of respiratory pathogens in the respiratory tract must include effective evasion mechanisms of different components of immunity. Evasion of complement activation is important for the survival of pathogens in tissues. One sign of the importance of complement in the rejection of pathogens is the fact that many of them have developed several strategies to evade complement activation (**Table 2**) (Du Clos 2008). The acquisition of soluble host complement regulatory factors is a clever way to camouflage from opsonization and phagocytosis. C4BP and FH are important regulators of the complement classical and alternative pathway, respectively. FH binds to C3b and accelerates the dissociation of the AP C3 convertase C3bBb. It is also a cofactor for factor I the cleavage of C3b (Blom et al. 2003). C4BP binds to C4b, and acts as a cofactor of factor I in the cleavage of C4b. It also accelerates the decay of the CP C3 convertase of C4b2a (Du Clos 2008).

Table 2. Complement evasion strategies by pathogens. Studied mechanism of *S. pyogenes* in bold.

	Method	Pathogen
Bacteria	Block C1, C3b deposition	*Streptococcus pneumoniae*
	Block MAC action	*Salmonella*
	Block AP activity by sialic acid capsule	*Streptococcus agalactiae* *Neisseria meningitidis group B*
	Limit access of C3 to C receptors by capsule	*S. pneumoniae* *N. meningitidis*
	Bind FH, C4BP to limit C activation	*S. pneumoniae* (Hic) ***S. pyogenes* (M-protein)** *Neisseria sp* *Borrelia sp*
	Use DAF, CD46 for attachment to cells	*S. pyogenes* (M-protein) *N. meningitidis* *Eschericchia coli*
	Use C receptors (CR3) for entry	*Mycobacterium tuberculosis*
Viruses	Express C regulatory proteins	HSV (glycoprotein C) Vaccinia (VCP)
	Use membrane receptors on regulators for entry	Epstein-Barr virus (CR2) HIV (CR3) Measles virus, adenovirus, herpesvirus 6 (MCP) Picornaviruses (DAF)
Parasites	Express C regulatory proteins	*Schistosoma* (CRIT) *Trypanosoma* (DAF-like protein)
	Take up C regulatory proteins	*Schistosoma* (DAF and CD59)
	Use C receptor for entry	*Leishmania* (CR1, CR3)

5.2.4.1 Group A streptococci and immune evasion

The group A streptococci *(Streptococcus pyogenes,* GAS*)* are extracellular gram-positive pathogens that cause a variety of infections. These range from mostly mild and common diseases (pharyngotonsillitis, impetigo, cellulitis, erysipelas, scarlet fever) to severe, life-threatening (up to 10% mortality) invasive infections like necrotizing fasciitis, streptococcal toxic shock syndrome or symptomatic bacteremia (Cunningham 2000). Even the mild and common superficial infections may cause remarkable morbidity as they sometimes lead to post-infectious immunological complications such as poststreptococcal glomerulonephritis, rheumatic fever and reactive arthritis (Cunningham 2000). Several potential virulence factors for GAS have been found. They include hyaluronic acid capsule, M-protein, C5a peptidase and serum opacity factor. The relevance of these factors to clinical disease has not, however, been clearly established (Jarva et al. 2003).

An important factor to GAS virulence is the fibrillar surface M-protein. It is a dimeric protein with a coiled structure. Its multiple functions include antiphagocytic activity, autoaggregation of bacterial cells, adherence to host tissues and intracellular invasion (Bisno et al. 2003). It has a distally projecting N-terminus containing a hypervariable region that has been used to define more than 100 M-serotypes (Facklam et al. 2002). GAS mutant strains lacking M-protein are readily phagocytosed, after being opsonized mainly by components of the classical pathway of C (Fischetti 1989; Bisno et al. 2003; Carlsson et al. 2003). The antiphagocytic property of GAS strains is usually serotype-specific and can only be transferred to types sharing a genetically homologous background (Kotarsky et al. 2000). Small epidemics caused by specific M-protein expressing strains have been described (O'Brien et al. 2002; Beres et al. 2004). Two of the most common serotypes causing invasive and toxic streptococcal infections are considered to be M1 and M3 (Colman et al. 1993; Cunningham 2000). GAS has several mechanisms to avoid host innate and adaptive responses and these are listed in **Table 3** (Kwinn et al. 2007).

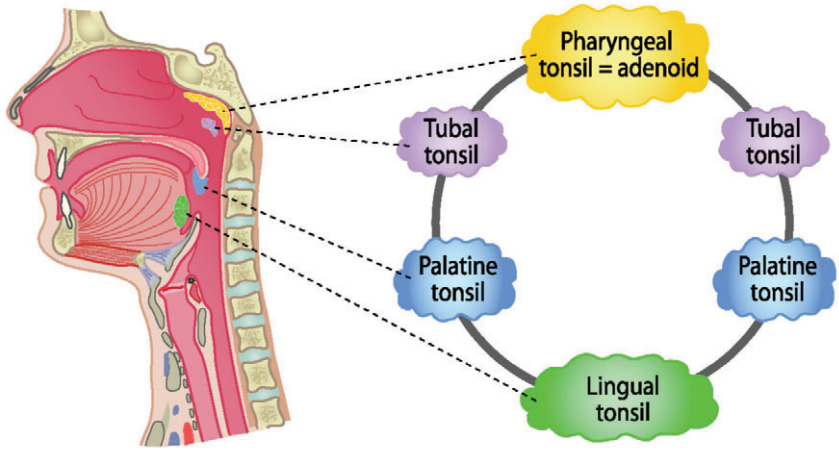
Table 3. Immune evasion mechanisms of Group A streptococci. Studied mechanism in bold.

	Factor	Mechanism
Phagocyte avoidance	Peptidase ScpA	Cleaves the complement-derived chemoattractant peptide C5a
	Serine protease ScpC	Degrades CXC chemokine IL-8
	DNAses	Prevent capture in DNA-based neutrophil extracellular traps
Inhibition of complement function	Hyaluronic acid capsule	Mimics common human matrix component Blocks opsonins from bacterial surface
	M-, M-like proteins and Sfb1	Recruit host matrix proteins (fibronectin, fibrinogen, collagen) to form protective coating
	M-protein	**Binds complement regulators C4BP and FH limiting C3b deposition**
	SIC	Interferes with formation of MAC
Antibody function inhibition	M-protein, Sfb1	Bind immunoglobulin nonopsonically via Fc domain
	Cysteine protease SpeB	Degrades immunoglobulins
Phagocytosis impairment	EndoS	Hydrolyses IgG glycans involved in Fcgamma recognition
	Mac-1, Mac-2	Bind to neutrophil Fc receptors, inhibit recognition of IgG on bacterial surface
	SIC	Impairs actin cytoskeletal arrangements required for bacterial uptake
Phagocyte lysis and promotion of apoptosis	Pore-forming Streptolysin S and O	Cytotoxic to neutrophils and macrophages
	Whole cells	Induce accelerated apoptosis program in human neutrophils
Resistance to phagocyte killing	SIC, SpeB proteolysis and D-alanylation of lipoteichoic acid	Interfere with host cationic antimicrobial peptide function
	Whole cells	Can escape the phagosome
	M-protein	Can block azurophilic granule:phagosome function

5.2.5 Innate immunity in adenotonsillar tissue

Pharyngeal, palatine, lingual and tubal tonsils, prominent parts of the Waldeyer's ring are usually small in the newborn, but grow in size during the first 1 to 5 years of life. They are immunologically most active between 4 and 10 years of age, and begin to decrease in size during puberty, yet their Ig production is maintained to old age (Wiatrak 1998).

Figure 3. Waldeyer's tonsillar ring. Tonsillar tissue localization in the upper airways.



Tonsillar tissue forms a torus-like formation surrounding upper airways, the so-called Waldeyer's ring (**Figure 3**). It consists of nasopharyngeal-associated lymphoid tissue (NALT), which is capable of follicle formation (Boyaka et al. 2000; Nave et al. 2001). Waldeyer's ring is an essential part of upper airway microbial defense acting both locally and systemically. In this secondary lymphoid tissue weak antigenic signals are eliminated and strong antigenic signals start the proliferation of antigen-specific B-cells. Tonsillar tissue functions actively in innate immunity and is also capable of regulating humoral adaptive immunity (Boyaka et al. 2000).

Adenotonsillar tissue is an important region for the induction of immunological responses. Microbes attach to the epithelial surface of tonsils covered by a viscous secretion containing several antimicrobial factors of innate immunity. Surface crypts allow the microbes to come into contact with macrophages and dendritic cells through specialized reticular epithelium. The adenoid tissue is well organized to T and B-cell areas and is capable of antigen uptake, processing, presentation, T/B-cell cooperation, maturation and differentiation (Boyaka et al. 2000; Nave et al. 2001).

Several potentially pathogenic bacteria are harbored in the human nasopharynx (*S. pneumoniae*, non-typeable *H. influenzae* (NTHI), *Neisseria meningitidis* and *Moraxella catarrhalis* (Forsgren et al. 1995). With in-situ DNA/RNA hybridization NTHIs has been demonstrated to reside and multiply intracellularly in subepithelial macrophages in the reticular crypt epithelium of human adenoid tissue (Forsgren et al. 1994). With DNA fingerprinting, NTHIs have been found to cause endogenous re-infections of acute otitis media after intracellular survival (Samuelson et al. 1995).

5.3 Adaptive immunity

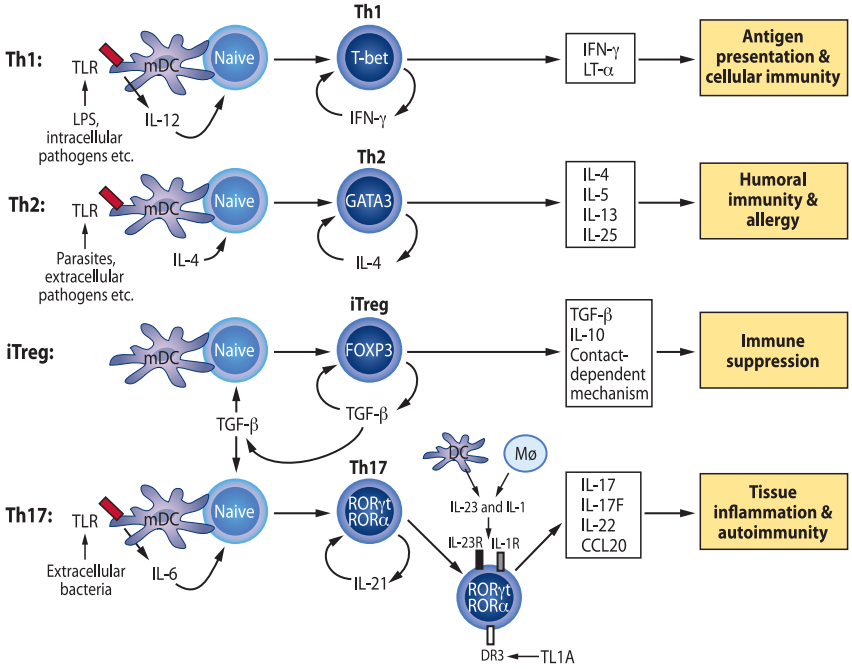
Adaptive immunity functions mainly through antibody action on antigen-carrying pathogenic substances or via T-cells recognizing peptides on MHC-molecules. Adaptive immunity is tightly regulated to ensure that damage is inflicted only to infective agents and harmful exogenous material. In autoimmunity, this control is breached and immune defense is activated against endogenous host structures causing damage to self (Janeway 2005).

5.3.1 Cellular adaptive immunity

Pathogen contact with APC triggers cellular adaptive immunity. These APCs, monocyte-derived Langerhans cells, DCs or macrophages can migrate actively between tissues and blood or lymphatic circulation. Activation occurs when PAMPs are recognized by PRRs and TLRs on the surface of APCs. APCs take in and process antigens into peptides and present them to naive CD4+ T-lymphocytes on class II major histocompatibility complex (MHC II) receptors with simultaneous cytokine excretion (Janeway 2005). Cytokine levels regulate naive T-cells' differentiation into different effector cells. IL-12 leads to T-helper 1 (Th1), IL-4 to T-helper 2 (Th2), tumor growth factor-beta (TGF-B) to inducible T-regulatory lymphocytes (iTreg), or T-helper 17 (Th17) development (Martinez et al. 2008). When activated, each T-cell has a different immunological function. Th1 promote the development of cytotoxic T-cells, natural killer cells and IgG-producing lymphocytes leading to antigen presentation and cellular immunity. Th2 promote humoral immunity by the development and maturation of IgG-, IgA- and IgE-producing B-lymphocytes, eosinophils and mast cells and also promote allergic and asthmatic responses.

iTregs lead to lymphocyte homeostasis, immune tolerance and regulation of immune responses and Th17 to tissue inflammation and autoimmune processes (Martinez et al. 2008; Zhu et al. 2008).

Figure 4. T-helper cell subset differentiation (modified from Martinez 2008).



LPS = lipopolysaccharide, mDC = mature dendritic cell, IL = interleukin (IL-4, IL-12, IL-21, IL-23 etc), Naive = naive CD4+ T-lymphocyte, T-bet = T-box expressed in T cells, LT = lymphotoxin (LTα), GATA3 = GATA-binding protein 3, FOXP3 = Forkhead box P3, TGF = tumor growth factor (TGFβ), ROR = retinoic-acid-receptor-related orphan reseptor (RORγt, RORα), DR = death receptor (DR3), TL1a = TNF-family cytokine, MØ = monocyte-macrophages, CCL = chemokine ligand (CCL20)

5.3.2 Humoral immunity

The adaptive humoral immune system is based on the ability of Igs to recognize and bind antigen through the highly variable V-region and interact with the conserved effector systems through constant C-regions. Two identical binding sites allow IgG to bind with a high avidity to antigens with repeating epitopes or to aggregates of antigen (Clark 1997). The binding of antibody to antigen may directly inactivate an infectious agent by blocking functional sites with receptor binding or enzymatic activity. However, most often, the antigen bound antibody interacts with other

components of the immune system (Janeway 2005). These interactions can lead to activation of complement through the classical pathway or to binding to receptors of various cell types. Both processes assist the opsonization of antigen, trigger inflammation and enhance an immune response against the infectious agent (Wingren et al. 2005).

5.3.2.1 Immunoglobulins

Immunoglobulins are glycoproteins with antibody function and found in all vertebrates. They exist as membrane bound receptors in B-lymphocytes, which after maturing to plasma cells secreted them as proteins. These constitute up to 10-20% of plasma proteins in mammals. Igs are mostly formed from two "heavy" and two "light" chains making symmetrical structural subunits with two identical antigen-binding sites (Wingren et al. 2005). The N-terminal domain, called variable or V-domain (Fab), gives rise to its specificity for its antigen and further to differences in antibody-binding affinity caused by somatic gene rearrangements and mutations (Janeway 2005). After pathogen recognition, during antibody response, the antigen specific B-cell clone secretes its B-cell receptor V-domain attached to a constant C-region domain (Fc). The elected C-domain defines the isotype ("class") of the antibody and further allows it to perform different effector functions. Different classes of immune effector cells carry different Fc receptors (FcR) and thus different antibody (sub)classes activate different effector cells (Clark 1997) .

In mammalians Igs exist in 5 classes: IgG, IgA, IgM, IgD and IgE. In humans, IgA is divided into two subclasses and IgG into four subclasses. Each B-cell produces an antibody with a single type of heavy chain associated with a single type of light chain (Wingren et al. 2005). IgG is the main mammalian Ig class. In humans, the four IgG subclasses show over 90% homology in the C region domains probably as a result of recent duplications in evolution. The half-life of IgG is much longer (3-4 weeks) as compared to the other Ig classes (IgA, IgM 3-7 days) and is inversely related to the total concentration of IgG in plasma (Clark 1997; Janeway 2005).

5.3.2.2 IgM, IgA and IgD

In addition to the cell bound IgM in the BCR, IgM is mostly found in the intravascular pool. It is the first antibody produced in the primary response and has a multimeric, most often pentameric structure. IgM is a potent binder of antigens and a strong C activator. It acts as an opsonin

and aids in the clearance of apoptotic cells (Ochs 2008). Natural antibodies are of IgM class. Constantly low IgM is associated with autoimmunity, hypersensitivity and with recurrent infections (Goldstein et al. 2006).

There are two forms of IgA, dimeric secretory IgA (sIgA) and monomeric serum IgA. Secretory IgA is produced locally in the mucosal surfaces, mainly in the gut associated lymphoid tissue (GALT). It serves as the first line of humoral defense and is capable of neutralizing viruses and toxins, opsonizing pathogens and blocking bacterial entry across mucosal surfaces (Woof et al. 2006). Serum IgA has a short half-life. Up to 60% of daily Ig production in humans is of IgA type. However, more than half of produced IgA is selectively transported to external secretions as sIgA. IgA has two subclasses IgA1 and IgA2. In serum IgA1/IgA2 ratio is 9:1 but in secretions this ratio is much more even. IgA is a poor opsonizer and complement activator. Antigen binding to IgA fails to initiate various inflammatory processes. On the contrary, to protect mucosal surfaces, it may inhibit excessive complement activation by complement fixing Igs. The function of serum IgA is poorly known, but it also may have anti-inflammatory functions (Jacob et al. 2008). IgA deficiency is the most common primary immunodeficiency, but most of its carriers are asymptomatic or suffer from only minor, noninvasive infections. In Finnish blood donors, its incidence was 2.5/1000 (Koistinen 1975). IgA deficiency has been associated with frequent mucosal infections, atopy, and certain immune diseases, like the celiac disease (Koistinen 1975; Cunningham-Rundles 2001; Woof et al. 2006; Latiff et al. 2007). Predisposing genes to IgA deficiency have recently been found (Sekine et al. 2007; Haimila et al. 2008).

IgD makes only 0.25% of the Ig population and its serum levels are low. IgD is mainly found as a membrane bound BCR and its precise function is unknown (Wingren et al. 2005).

5.3.2.3 IgG and IgG subclasses

IgG is the most abundant circulating antibody class; it composes 70-75% of the total serum Ig. It is evenly distributed to the intra- and extravascular pools. Most anti-protein antibodies are of IgG1 and IgG3-type, whereas IgG2 and IgG1 are effective against pathogens with polysaccharide antigens. Ig subclasses IgG1-4 differ from each other in their effector functions (Pan et al. 2000). IgG1 is most effective in activating complement and in triggering cell-mediated cytotoxicity (antibody-dependent cell-mediated cytotoxicity, ADCC). IgG3 does the same, but with somewhat weaker efficiency than IgG1. IgG1 function is more effective when anti-

gen concentrations are higher; IgG3 works relatively better at low antigen concentrations. Due to the shorter half-life of IgG3, IgG1 levels in plasma are 10-20 times higher than IgG3 levels though more IgG3 is produced. IgG1 and IgG3 both activate the CP (Janeway 2005). The production of IgG1 and IgG3 is thought to be enhanced by Th1-dominant inflammatory responses. IgG1 and IgG3 are capable of all antibody mediated effector functions (Pan et al. 2000).

IgG2 and IgG4 appear during the secondary immune response and their main functions is to neutralize extracellular antigens. IgG2 is important in anti-polysaccharide antibody responses. It is able to trigger complement lysis only at very high concentrations and does not trigger ADCC. IgG4 does not activate C nor induce ADCC under any conditions. IgG2 production is enhanced by Th2 dominant inflammatory responses, and it is the most abundant subclass to react with carbohydrate antigens. IgG4 production is enhanced in response to prolonged exposure to mucosal antigens causing a Th2 type response, such as during simultaneous exposure to multiple allergens or helminth infection (Clark 1997).

5.3.2.4. IgG subclass deficiencies

Individuals with low IgG subclass levels are common and they are frequently asymptomatic (Maguire et al. 2002). In children, there is a 3:1 male to female preponderance in low subclass levels. Boys have more frequently low IgG1 levels (Lacombe et al. 1997). Low IgG2 and abnormal vaccine responses are more frequent in children. After puberty, low IgG1 and IgG3 levels are seen more often, especially in females. Selective IgG1 deficiency without low levels of other subclasses is associated with mostly moderate upper respiratory tract infections and sinusitis caused by *S. pneumoniae* or *H. influenzae*. Only 12% of symptomatic IgG1 deficient individuals get severe infections (Lacombe et al. 1997). Severely symptomatic patients with low IgG1 (and thus almost always low IgG) levels and impaired responses to polysaccharide antigens are diagnosed to have common variable immunodeficiency (CVID) or specific antibody deficiency (SAD). These patients are candidates for subcutaneous or intravenous Ig substitution therapy, if prophylactic antibiotics are not sufficient or serious end-organ damage is found (Buckley 2002; Maguire et al. 2002; Bonilla et al. 2005; Orange et al. 2006; Provan et al. 2008).

The clinical significance of low IgG3 level is controversial. It is associated with frequent, but mild URTI, bronchitis, bronchopneumonia, bronchial asthma, and erysipelas episodes and with recurrent herpes simplex vi-

rus infections (Oxelius et al. 1986; Morgan et al. 1988; Aucouturier et al. 1994; Seppänen et al. 2006). These patients rarely have impaired responses to vaccines.

Individuals with selectively low IgG2 levels are also usually asymptomatic. In children, IgG2 deficiency is associated with recurrent sinopulmonary infections caused by *S. pneumoniae, H. influenzae* and *M. catarrhalis*. The association is strong only if associated with an impaired vaccination response (Buckley 2002; Bonilla et al. 2005). Symptomatic selective IgG2 or IgA deficiencies in childhood may progress to CVID, but Ig levels and vaccination responses may also normalize and patients become asymptomatic (Bonilla et al. 2005; Orange et al. 2006).

A combination of low levels of IgG1 and IgG3 is frequently seen in patients with nonatopic or atopic bronchial asthma with sinopulmonary symptoms (Lacombe et al. 1997). This phenomenon seems to be associated with Th2 dominant immune responses (Avery et al. 2008). IgA deficiency is a predisposing factor to atopic and certain immune diseases, like celiac disease (Haimila et al. 2008). Sinopulmonary symptoms caused by atopic diseases together with recurrent sinopulmonary infections associated with IgG1, IgG3 and IgA deficiencies in the same patient constitute a diagnostic dilemma (Lacombe et al. 1997; Cunningham-Rundles 2001; Buckley 2002; Wood et al. 2007).

5.3.2.5 IgE and allergy

IgE mainly exists bound to its receptors in various cells and is present in only very low quantities in serum. IgE makes only about 0.002% of the total Ig pool and 50% of it is found intravascularly with a half-life of 1-5 days. IgE is involved in parasite and allergen-specific Th2-dominant responses (**Figure 4**) (Galli et al. 2008). The main actions of IgE are mediated through basophils, mast cells and eosinophils, where cross-linking of IgE FcεRI- receptors causes the release of inflammatory mediators, proteases and cytokines [histamin, leukotrienes and platelet activating factor (PAF)] from the secretory granules (Prussin et al. 2006). In addition to this immediate IgE-mediated hypersensitivity reaction, mast cell activation contributes to the delayed hypersensitivity reaction by releasing histamine, lipid mediators and cytokines. Eosinophil activation also contributes to the allergic late reaction by releasing basic proteins, leukotrienes and PAF. Besides allergy, there are several diseases causing high levels of IgE: parasitic and some viral infections, hematological malig-

nancies, autoimmune disorders, hyper-IgE syndromes and several forms of combined immunodeficiencies (Prussin et al. 2006; Galli et al. 2008; Pien et al. 2008).

IgE does not activate complement. Interestingly, activation of complement receptors C3aR and C5aR by anaphylatoxins C3a and C5a acts synergistically with IgE-mediated responses (Prussin et al. 2006).

Hypersensitivity causes objectively reproducible symptoms or signs, initiated by exposure to a defined stimulus at a dose tolerated by normal subjects. Atopy is a personal or familial tendency to produce IgE antibodies in response to low doses of allergens, usually proteins, and to develop typical symptoms such as asthma, rhinoconjunctivitis, or eczema/dermatitis. Allergy is a hypersensitivity reaction initiated by immunologic mechanisms (Johansson et al. 2001).

Allergens are usually proteins, or rarely inorganic substances that first have to bind to a host protein to form a hapten before they become allergens (Johansson et al. 2001). Allergens can cause hypersensitivity reactions in several ways. If an allergen binds to IgE and induces histamine release from effector cells, it is considered a true allergen. Most respiratory (pollens, dusts) and some of the food allergens (e.g. egg, milk) belong to this group. In these cases, skin prick testing and tests to detect specific IgE against allergens are positive. If the allergen does not bind to IgE, but is capable of inducing histamine release, it is considered to be a nonspecific histamine liberator. Analgesic morphine or some fruits (kiwi) belong to this group. If the allergen does not bind IgE and does not release histamine it is considered to function through cell-mediated or other mechanisms. Metallic nickel and some artificial scents belong to this group; these can be assessed with epicutaneous testing. Finally, allergic symptoms can be caused by a substance not binding IgE, but itself containing histamine or other biogenic amines (Jansen et al. 2003). The meat of a marine predatory fish species (e.g. tuna), allowed to warm up after catching belong to this group. Some wines and cheeses may also contain biogenic amines (Budde et al. 2003).

The clinical diagnosis of allergy is confirmed when either allergen-specific IgE is found from the serum or when skin prick testing (SPT) is positive, in conjunction with compatible symptoms (allergic rhinoconjunctivitis, bronchial asthma or cutaneous or gastrointestinal allergic symptoms). There are, however, exceptions making the diagnosis more challenging. Specific IgE can be found and/or SPT can be positive in atopic patients

even without allergic symptoms. Conversely, in patients who have developed clear seasonal allergic symptoms both specific IgE and SPT testing may be falsely negative due to only local IgE production and/or IL-10 mediated suppression of IgE production (Heaton et al. 2005). Also allergen extract quality, seasonal variations in IgE synthesis, patient's age and medication may influence the reliability of allergy testing (Bousquet et al. 2008).

The nose is an important route for sensitization to respiratory allergens (Vinke et al. 1999). CD1a+ Langerhans cells (APCs) are found in the nasal mucosa. These are capable of presenting allergens to T-cells in the lamina propria and lymphoid tissue (Fokkens et al. 1992). Most of antigen presentation occurs in the regional draining lymphoid tissue composed by the Waldeyer's ring (Winther et al. 1994). In the adenoid tissue, CD1a-positive cells are found in larger numbers in children with allergic disease than in non-allergic children. A similar increase in APCs has been seen in nasal mucosa of allergic patients after allergic challenge. Larger numbers of eosinophils, important effector cells in allergic reactions, have also been found in the adenoid tissue of allergic children when compared to controls. These cells that normally are seen in the mucosal lining of allergic "shock organs" (airways, gut) are rapidly recruited and activated after allergen stimulation (Godthelp et al. 1996).

5.4 Upper airway diseases

5.4.1 Sinonasal disease definitions

Rhinosinusitis (R) is the common term for concurrent rhinitis and sinusitis. This disorder is common in both adults and in children. The definition of rhinosinusitis has changed during the last decade. The definition by Lanza and Kennedy recognizes acute recurrent rhinosinusitis, but not nasal polyposis as this is considered to be a different disease (Lanza et al. 1997). In the European Academy of Allergology and Clinical Immunology Task Force document (European Position Paper on Rhinosinusitis and Nasal Polyps, EPOS), new evidence-based definitions for these sinonasal disorders were recently proposed for both clinical and research purposes (Fokkens et al. 2007). In this definition, NP is considered to be a subgroup of chronic rhinosinusitis. **Table 4** shows the main differences between these definitions of adult R (Lanza et al. 1997; Fokkens et al. 2007).

WHO organized a workshop on Allergic Rhinitis and its Impact on Asthma (ARIA) to make an evidence-based approach to the definitions, impact, diagnostics and treatments of these conditions (Bousquet et al. 2008).

5.4.2 Rhinosinusitis in adults

Common cold, acute viral R, is estimated to affect adults 2-5 times/year and school children 7-10 times/year (Mackay 2008). Most colds are self-limiting and mild. Rhinoviruses (24%, but up to 80% in seasonal epidemics) and influenzaviruses (11%) are the main causative agents, but more than 200 viruses or viral serotypes have been described in acute URTI with recent sensitive detection methods (Monto 2002).

Limited data about acute rhinosinusitis (ARS) and CRS epidemiology exist since the definitions and patient selection criteria have not been uniform. Only 0.5-2% of acute viral URTI have been estimated to become complicated by a bacterial infection, but this estimate is subject to debate as the diagnosis of bacterial infection is often impossible to make without invasive procedures. Acute viral R commonly precedes bacterial infection (Heikkinen et al. 2003). Most common bacterial species isolated in ARS are *S. pneumoniae* and *H. influenzae* (Poole 2004). Commonly, antibiotics are prescribed for ARS symptoms although bacterial sinonasal infection is known to be far less common than a prolonged viral infection (Small et al. 2007).

Table 4. Rhinosinusitis definitions 1997 and 2007.

Definitions from the Task Force on Rhinosinusitis sponsored by American Academy of Head and Neck Surgery 1997							
DEFINITION	**DURATION**	**SYMPTOMS**		**SIGNS IN ENT-EXAMINATION**		**IMAGING**	**SEVERITY**
ACUTE	≤4 weeks	**Strong**: 2 major or 1 major and ≥2 minor **Suggestive**: 1 major or ≥2 minor [1)]	**OR**	Purulence in nasal cavity		-	-
SUBACUTE	4-12 weeks	Same as chronic					
RECURRENT ACUTE	≥ 4 episodes/ year, each ≥7-10 days	Same as acute, no symptoms of chronic. No symptoms between episodes				-	-
CHRONIC	≥12 weeks	**Strong:** ≥2 major factors, 1 major and 2 minor factors **Suggestive**: 1 major or ≥2 minor [1)]	**OR**	Purulence in nasal cavity		-	-
ACUTE EXACERBATION OF CHRONIC		Sudden worsening of chronic, with return to baseline after treatment				-	-
Definitions from European Position Paper on Rhinosinusitis and Nasal Polyps 2007							
ACUTE/ INTERMITTENT	<12 weeks with complete resolution of symptoms	Sudden onset of [2)]	**AND**	[3)]	**AND/ OR**	[4)]	Visual Analogue scale: Mild 0-4 Moderate 4-7 Severe 8-10
CHRONIC (CRS)	>12 weeks without complete resolution of symptoms	[2)]	**AND**	[3)]	**AND/ OR**	[4)]	
CRS (major finding)		[2)]	**AND**	No visible polyps in middle meatus, if necessary following decongestant			
NASAL POLYPOSIS (subgroup)		[2)]	**AND**	Polyps bilateral, endoscopically visualised in middle meatus			

[1)] Major factors: facial pain/pressure (alone does not constitute a suggestive history for rhinosinusitis in the absence of another major nasal symptom or sign), facial congestion/fullness, nasal obstruction/blockage, nasal discharge/purulence/discolored postnasal drainage, hyposmia/anosmia, purulence in nasal cavity on examination, fever (acute rhinosinusitis only). Minor factors: headache, fever (all nonacute), halitosis, fatigue, dental pain, cough, ear pain/pressure/fullness. Fever in acute sinusitis alone does not constitute a strongly suggestive history for acute in the absence of another major nasal symptom or sign.

[2)] Inflammation of the nose and the paranasal sinuses characterised by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip), ± facial pain/pressure, ± reduction or loss of smell.

[3)] Endoscopic signs of polyps and/or; mucopurulent discharge primarily from middle meatus and/or; oedema/mucosal obstruction primarily in middle meatus.

[4)] Mucosal changes within the ostiomeatal complex and/or sinuses in sinus CT

Various microbial, host and environmental factors have been suggested to predispose to R. The role of bacteria is still a matter of debate even though the microbiology of the middle nasal meatus and sinuses has been thoroughly studied. However, both aerobic and anaerobic bacteria have been recovered from the affected and non-affected sides of unilateral R (Bhattacharyya 2005). Anaerobic bacteria are more commonly found in odontogenic sinusitis. Antibiotics and nasal steroids have been shown to be equally effective in the treatment of ARS in adults (Meltzer et al. 2005).

Fungi have also been found in both healthy and diseased human osteomeatal area (Fokkens et al. 2007). A range of fungal sinonasal diseases ranging from non-invasive fungus balls to invasive disease have been described (Schubert 2001). A concept of fungal antigens causing IgE or IgG3 mediated inflammation in the sinuses has been proposed. However, no causality between the presence of fungi and CRS has been established (Fokkens et al. 2007). The use of systemic or topical antifungal agents does not consistently help patients with CRS (Weschta et al. 2004; Ebbens et al. 2006).

Until now, most of the microbiology of the respiratory infections has concentrated on studying the non-attached, planktonic bacteria. When attached to surface some bacterial species may produce an extracellular matrix called biofilm that protects them from both host immune response and antibiotics. Recently, more evidence has been found about the role of microbial biofilms as a cause of otorhinolaryngolocial infections such as otitis media with effusion (Macassey et al. 2008). Biofilms have also been suggested to be important in treatment-refractory CRS (Hunsaker et al. 2008).

Cigarette smoking has been suggested to associate with a high prevalence of CRS (Chen et al. 2003), but no convincing evidence exists for the etiologic role of pollutants in CRS or NP (Fokkens et al. 2007).

5.4.3 Nasal polyposis

Aspirin (ASA) sensitivity is a non-allergic syndrome caused by abnormal metabolism of arachidonic acid metabolites. It involves acute allergic reactions to non-steroidal anti-inflammatory drugs metabolized through cycloxygenase (COX) pathways. Aspirin sensitivity can cause a treatment-resistant form of bronchial asthma and commonly also CRS and NP (Fokkens et al. 2007). CRS with NP is seen in 36-96% of patients with aspirin sensitivity, and 96% of them have radiologic abnormalities

in the paranasal sinuses (Szczeklik et al. 1977; Fahrenholz 2003). A high incidence of human leucocyte antigen (HLA) A1 and B8 has been reported in patients with asthma and ASA sensitivity (Moloney et al. 1980). Contradictory reports on association of allergy and NP have been published (Fokkens et al. 2007).

Severe primary antibody deficiencies frequently present with severe recurrent R (RRS) with or without NP (Wood et al. 2007; Herriot et al. 2008). The most common minor defects in adaptive immunity, IgA deficiency and low IgG subclass levels may predispose to CRS and RRS (Baumann et al. 2007). However, the clinical significance of abnormal IgG subclass levels in CRS patients remains unclear (Wood et al. 2007).

In addition to relatively rare primary antibody deficiencies like X-linked agammaglobulinemia (XLA, caused by intracellular tyrosine kinase mutations), cystic fibrosis and primary ciliary dyskinesia are the only genetic abnormalities linked to CRS. Cystic fibrosis is one of the most common autosomal recessive disorders elsewhere in the Caucasian population and is caused by mutations in the *CFTR* gene in chromosome 7 (Riordan et al. 1989). The mutations in *CFTR* have been reported to cause CRS also in the general population (Wang et al. 2000). Cystic fibrosis is, however, rarely seen in Finland (Hytönen et al. 2001).

There is a strong hereditary component in NP in family studies. The few existing twin studies suggest additional influence of environmental factors. In linkage analysis and association studies some candidate gene polyporphisms have been associated with certain phenotypes of NP. In the gene coding IL-1alpha (*IL1A*) a single G to T polymorphism in exon 5 at +4845 was associated with a reduced risk of developing NP (Karjalainen et al. 2003). In another study IL-4 gene polymorphism (*IL4*/590 C-T) in the Korean population was associated with protection against NP (Park et al. 2006). Several correlations between HLA-alleles and NP have been presented (HLA-A74, HLA-DR7-DQA1*0201, HLA-DR7-DQB1*0202, DQB1*0201, HLADRB1*03, HLA-DRB1*04), but these are dependent on the ethnic background of the population studied (Fokkens et al. 2007).

Local host factors, such as sinonasal anatomical abnormalities have not convincingly been found to be in causal relationship with CRS although sinonasal complaints often resolve with surgery (Fokkens et al. 2007).

5.4.4 Airway allergy

Allergic disorders have been defined as an epidemic of the Western countries (Haahtela et al. 2008). Both allergic rhinitis (AR) and asthma are systemic inflammatory conditions and often co-exist. This has lead to the concept of "United Airways" where both conditions are to be evaluated and managed in each patient (Bousquet et al. 2008).

Allergic rhinitis and bronchial asthma have been associated with CRS. The role of allergy in the development of CRS is still unclear, although epidemiological data shows an increased prevalence of allergic rhinitis in patients with CRS. There is a problem in evaluating the cause-effect relationship as the symptoms of CRS and allergic rhinitis partly overlap (Ryan 2008). Allergy is a poor prognostic factor in postoperative results in some, but not all studies (Meltzer et al. 2004). Abnormalities in the mucosa of paranasal sinuses are seen in sinus imaging in asthmatics, but this may reflect either allergic inflammation or infection (Fokkens et al. 2007).

In adult Finnish population, the prevalence of physician diagnosed AR or allergic conjunctivitis was 41.9% and of asthma 6.9% (Pallasaho et al. 2006). At least one skin prick test was positive in 46.9% of all subjects. In young adults (age 26-39) 56.8% were sensitized to at least one allergen, 24% to at least four. Sensitization to multiple allergens was associated with high prevalence of asthma, AR or conjunctivitis, and wheeze.

5.4.5 Tonsillar diseases in children

Usually a larger area of the pharynx than the tonsils themselves is affected during episodes of tonsillitis/tonsillopharyngitis. These diseases are commonly caused by respiratory viruses, but bacterial involvement is not unusual. Typical bacteria found in tonsils are beta-hemolytic streptococci of the A, B, C or G-group, *H. influenzae* or staphylococci. Also yeasts and *Actinomyces* have been found in tonsils, but their pathogenicity is largely unknown (Wiatrak 1998). Altogether tonsils are known to harbor several pathogenic and non-pathogenic microbes, yet their association to tonsillar diseases is not always clear. In acute tonsillitis, GAS are considered a significant pathogen due to their potential for systemic invasive infections and postinfectious complications (Bisno 1996; Wiatrak 1998).

In recurrent or prolonged tonsillitis, the immunogically active reticular epithelial cells are replaced by squamous epithelium. This weakens antigen transport, B-cell activation, and Ig production and probably makes an individual more susceptible to new episodes of tonsillitis (Brodsky et al. 1993). Recurrent tonsillitis is established if episodes recur 7 times per year or 5 times in consecutive years (Paradise et al. 1984).

Chronic tonsillitis is a poorly defined clinical diagnosis and is based on typical findings in pharyngeal inspection and possibly positive streptococcal culture. Patient commonly has prolonged pharyngeal pain, enlarged and/or painful cervical lymph nodes, halitosis, ceratosis or purulent discharge from tonsillar crypts (Darrow et al. 2002).

In tonsillar hyperplasia, enlarged tonsils are seen due to hypertrophic lymphoid tissue. This is seen together with recurrent and chronic tonsillitis (recurrent tonsillitis with hyperplasia, RT-TH) but also with no history of infections (idiopathic tonsillar hyperplasia, ITH). RT-TH is considered to be a subtype of recurrent tonsillitis and ITH is possibly a different disease entity. Tonsillar hyperplasia may cause, depending on the degree of the hyperplasia, constant oral breathing, malocclusion, disturbances in craniofacial growth, eating disorders or speech impairment. Night-time problems may include snoring, sleep-related breathing disorder, obstructive sleep apnea syndrome, enuresis and restless sleep with daytime hypersomnolence or behavioral disorders. Reliable epidemiological data about the incidence and prevalence of tonsillar diseases is limited (Mattila et al. 2001; Darrow et al. 2002).

Operations of pharyngeal and palatine tonsils are still among the most common operations in children. The numbers of tonsillar operations have decreased lately in several Western countries. In children, until the age of 10, the most common indication for tonsillar operation is hyperplasia, in teenagers peritonsillar abscess and in 20-year-olds chronic tonsillitis (Mattila et al. 2001; Vestergaard et al. 2007). Only three systematic reviews about tonsillectomies have been made. The first one gives high rated evidence-based recommendation for outpatient surgery in properly selected children (Brigger et al. 2006). The second one showed that antibiotics reduced fever and the duration of halitosis and marginally the time to resume normal activity after operation, but had no effect on pain scores or the need for analgesia (Dhiwakar et al. 2008). Instead, an increased risk for adverse events due to antibiotics was observed. The third review showed that electrodissection increases pain in patients with no difference in postoperative hemorrhage rates (Leinbach et al. 2003).

5.5 The role of sex in immunity and respiratory diseases

Females are more immunocompetent than males. This sexual dimorphism is a common feature in vertebrates and even in a number of invertebrates (Nunn et al. 2009). In humans, it has long been known that females and males also behave differently when immunological reactions and susceptibility to infections are considered. Females produce more vigorous humoral and cellular reactions to infections and are more resistant to certain bacterial infections. On the other hand, the risk for autoimmune disorders (such as multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus and thyroiditis) is higher in females (Whitacre 2001; Bouman et al. 2005). This dichotomy is only partly due to the reproductive hormones. The interest for the study of sexual dimorphism has spread from autoimmune diseases to infectious diseases and the information about possible causative factors for these differences has increased.

5.5.1 Innate immunity

Effects of sex-hormones to the monocyte counts are well known. Female sex-hormones induce the release of monocytes from the bone marrow during luteal phase and pregnancy (Bain et al. 1975). Estrogen, and possibly progesterone also, seems to decrease monocyte numbers probably by inducing mitotic arrest and apoptosis (Thongngarm et al. 2003).

Tumor necrosis factor (TNF) is an important mediator of proinflammatory responses. In males, monocytes produce more TNF, but in vitro no effect of testosterone on the production of TNF has been seen. In females, reproductive phase influences the TNF production leading to higher plasma levels and stronger reactions to endotoxin stimulation in the luteal phase (Brannström et al. 1999; Bouman et al. 2001). Interestingly, also after the menopause, with low levels of circulating female sex hormones, TNF production is higher than in men, suggesting that factors other than hormones affect monocytes (Bouman et al. 2004; Bouman et al. 2004). When LPS stimulated TNF levels were measured in the peripheral blood a weaker response in females was found. This difference was partly HLA-independently sex-related, partly HLA-related and partly due to unknown reasons (Moxley et al. 2002).

5.5.2 Adaptive immunity

Gonadal hormones (estrogens, progesterone and testosterone) can modulate immunity. Estrogen receptors are found in immune cells. Estrogen has a biphasic dose effect with low levels and high levels inhibiting specific immune reactions. After a challenge by an infective agent or antigen, females are more likely to develop a systemic Th1-type response than males. An exception is pregnancy, when Th2-type systemic responses prevail (Moxley et al. 2002). Progesterone promotes the development of Th2-cells and antagonizes the Th1-response. The Th1 phenotype in peripheral blood lymphocytes is slightly inhibited by estrogens and stimulated by androgens (Paavonen 1994; Giltay et al. 2000). Females have a more vigorous immune response with more abundant antibody production and strong cell-mediated immunity after immunization (Bouman et al. 2005). Females also have more pituitary hormones (prolactin, growth hormone) that enhance autoimmunity. Testosterone has anti-inflammatory properties and is immunosuppressive in several animal models of autoimmunity. Based on a murine model, it has been suggested that sex chromosomes and sex hormones may compensate for each other in causing immunostimulation and -inhibition (Palaszynski et al. 2005).

The actual cellular and molecular mechanisms of sex hormone actions on immune system are not yet clear (May 2007). Fluctuations in hormone levels during pregnancy affect the course of autoimmune diseases. Sex hormone treatments for autoimmune diseases have been tried with sometimes promising results (Schmidt et al. 2006). Females have higher levels of circulating IgA than males, but this difference is seen only after puberty: this suggests that gonadal hormones may influence IgA production (Bouman et al. 2005). Females also have higher levels of circulating IgM than males. This difference is most apparent in puberty, but can be seen already in prepubertal children suggesting a mechanism not related to sex-hormones (Butterworth et al. 1967).

5.5.3 Allergic diseases

The risk for several allergic diseases in childhood has been recognized to be greater in males than in females (Jensen-Jarolim et al. 2008). However, during the last years the difference has diminished due to an increase of allergic conditions which has been greater in girls (Osman et al. 2007). In boys a higher risk than in girls for bronchial (atopic) asthma, food allergy and atopic status confirmed by SPT or serum specific IgE for allergens has been seen in several studies (Emmett et al. 1999; Pumphrey et al. 1999; Govaere et al. 2007; Jacobson et al. 2008). In adolescence this

is reversed, and adult females suffer from more respiratory allergies, food allergy and asthma (Chen et al. 2008). This change may at least partly be caused by sex-hormones. Estradiol has been shown to increase mast cell activation and allergic sensitization in a rodent model (Yamatomo et al. 2001; Melgert et al. 2005). Progesterone supresses histamine release but potentiates IgE formation (Vasiadi et al. 2006; Mitchell et al. 2007). A male sex hormone, dehydroepiandrosterone, downregulates Th2 cytokine production but does not affect IgE levels (Sudo et al. 2001; Yu et al. 2002).

5.5.4 Respiratory infections

Both the incidence and the severity of respiratory infections are different in females and males. Generally, females get more infections of the upper respiratory tract, especially sinusitis and tonsillitis (**Table 5**) (Falagas et al. 2008). Males are more often suffering from otitis media, laryngeal croup and LRT infections. In females the course of the disease is usually less severe. In contrast, males suffer from more complicated diseases and have a higher mortality, especially due to community-acquired pneumonia.

5.5.4.1 Sinonasal infections

Females appear to catch common cold and acute sinusitis more often than males (Gonzales et al. 1997; Lieu et al. 2000; Stalman et al. 2001; Chen et al. 2003; Shashy et al. 2004; Xatzipsalti et al. 2005; Barrett et al. 2007). Recurrent R (Bhattacharyya et al. 2005; Poetker et al. 2008) and CRS without polyposis (Collins et al. 2002; Shashy et al. 2004) occur more often in females than in males. On the other hand, CRS with polyposis is more common in males (Fokkens et al. 2007).

5.5.4.2 Tonsillar infections

In several studies acute/recurrent tonsillitis has more commonly been seen in female than male patients (Thorp et al. 2000; Lin et al. 2003; Kvestad et al. 2005). In males, peritonsillar abscesses were more common (Matsuda et al. 2002).

In a very interesting study from Denmark, 153 212 tonsillectomies performed between years 1980 and 2001 were reviewed (Vestergaard et al. 2007). The results showed that there were two peak ages for tonsillectomy. The first peak was at 4 years of age. For boys, 9.7 operations per 1000 person years were performed. For girls, the figure was 6.9. Male to female ratio was 1.41. The second peak was during adolescence, in girls at 16 years (8.6/1000 person years operated) and in boys at 17 years (3.1/1000). Male to female ratio was 0.36. From 8 years to 40 years of age females were operated more often, but after 40 years of age the operation rate was smaller in females (0.13 females vs. 0.16 males/1000 person years operated). The overall male to female ratio for all ages was 0.81/1000 person years. Similar results have been obtained from other studies but the reasons for these sex differences have remained unknown (Freeman et al. 1982; Mattila et al. 2001).

Table 5. Sex-associated differences in the incidence of upper respiratory tract infections.

	Age (years)	Subjects	Number of patients	Male to female ratio	Reference
Common cold (Flu)	18-74	Common cold	205	0.47	(Barrett et al. 2007)
	1-14	Acute symptoms	221	0.90	(Xatzipsalti et al. 2005)
	>18	URTI[1] patients	548	0.59	(Gonzales et al. 1997)
Sinusitis					
Acute	15-65	GP[2]	177	0.51	(Stalman et al. 2001)
Chronic	0-94	Patients (primary care)	2405	0.48	(Shashy et al. 2004)
	>12	Patients (survey)	3768	0.49	(Chen et al. 2003)
	>17	Patients (survey)	5848	0.66	(Lieu et al. 2000)
Tonsillitis/Pharyngitis					
Subclinical	5-15	School children, BHS[3]	749	0.39	(Gupta et al. 1992)
Acute	1-15	GAS+[4]	252	1.29	(Lin et al. 2003)
	adults	Hospital admission	111	0.54	(Thorp et al. 2000)
	>14	Tonsillectomy		0.31	(Thorp et al. 2000)
	All	Tonsillectomy	7250	0.69	(Thorp et al. 2000)
Recurrent	>18	Twins	9479	0.62	(Kvestad et al. 2005)
Chronic					
Tonsillectomies	All	All operated 1980-2001	153212	0.81	(Vestergaard et al. 2007)
Peritonsillar abscesses	7-92	Patients (tertiary care)	724	2.96	(Matsuda et al. 2002)

[1] URTI= Upper respiratory tract infection, [2] GP=General practitioner, [3] BHS=Beta-haemolytic streptococci, [4] GAS+=Group A streptococci culture +

5.6 Rhinoviruses

Common cold i.e. an acute viral URTI is the most common illness in humans, occurring twice as frequently as the next common condition. URTIs are caused by several different respiratory viruses, human rhinoviruses (HRVs) being the most common (Puhakka et al. 1998; Heikkinen et al. 2003; Arnold et al. 2008; Peltola et al. 2008). HRV causes up to 34 % of all URTIs, and during its main peak season (September-October following the opening of schools) even up to 80% and during the sec-

ondary peak season (April-May) up to 30% of URTIs. Although not an indication, HRVs are the most common reason for prescribing antibiotics for respiratory illness, exceeding even bacterial infections (Rotbart et al. 2000). Another very important fact in HRV morbidity is that it is the most common cause for wheezing in children and asthmatics (Rotbart et al. 2000; Monto 2002; Papadopoulos et al. 2002; Jartti et al. 2004; Jacques et al. 2006).

Over 100 serotypes of human rhinoviruses (HRVs) have been recognized. HRV is an RNA virus with adenine and uracil (A+U) rich capsid. This capsid of most of the HRVs interacts with the amino-terminal domain of the 90 kDa adhesion molecule ICAM-1/CD54 (Greve et al. 1989; Staunton et al. 1989; Tomassini et al. 1989; Rossmann et al. 2000). Receptor binding destabilizes the capsid and initiates uncoating. A minor group of HRVs use members of host low-density lipoprotein receptor (LDLR) family for attachment and use a different destabilization method for uncoating of the virus.

The transmission of HRVs occurs both directly via airborne aerosol and large droplets and through indirect contacts with contaminated secretions. Both routes are important, and therefore hand disinfection can only give partial protection. Crowding increases transmission. URTI hits especially little children, with a yearly incidence of 5-7 in 1-2 year old children. A decrease in incidence after the age 5-9 years is seen followed by a slight increase especially in females at 20-29 years. This may reflect an exposure to young children known to be able to infect their parents (Lidwell et al. 1951; Monto 2002). URTI has been shown to be more frequent in males up to 3 years of age and in girls thereafter (Monto 1994).

Rhinovirus RNA is taken into the cell mostly through the ICAM-1 (CD54) receptor. This cell surface glycoprotein is present on antigen-presenting cells, lymphocytes, eosinophils, mast cells, submucosal glands, airway smooth muscle cells, and epithelial cells. The function of ICAM-1 is to regulate leukocyte trafficking and accumulation at sites of inflammation. Rhinovirus infection upregulates the expression of ICAM-1 and induces a rapid increase in serum IgE levels. In contrast, HRV has not been found to elevate antigen-specific IgE levels in allergic subjects (Skoner et al. 1995).

Symptoms of HRV infections develop within days of virus inoculation (Holmes et al. 1976). Sneezing, nasal disharge (rhinorrhea), nasal congestion/blockage, sore or irritated throat, headache, cough, feeling of fever and malaise are common symptoms (Mackay 2008). Also loss of taste and smell, hoarseness, body aches and pains, feeling of pressure in the ears and sinuses, mild burning feeling in the eyes, anorexia and loose stools

have been reported (Mackay 2008). Even a self-limiting asymptomatic sinusitis may be associated with common cold, most often caused by acute HRV infection (Puhakka et al. 1998). Fever is more common in children (Miller 1955; Dick et al. 1967). In children, even uncharacteristic irritability, disturbed sleep patterns and feeding difficulties due to snuffles can be seen (Mackay 2008). The treatment of HRV infections is most commonly symptomatic. Decongestants, analgesics, antihistamines, nasal steroids and antitussives have been used, but no HRV-targeted medication is commercially available at the moment.

HRV has been detected in the lower respiratory tract (LRT) (Papadopoulos et al. 2000), causing acute wheezing episodes in children, especially among males and during the first year of life (Mackay 2008). Also in adults over 40 years of age respiratory infections caused by HRV associate with longer and more frequent LRT illnesses causing nearly two thirds of the total burden of respiratory illness (Monto et al. 1987). HRVs have long been associated with AOM (Arola et al. 1990; Vesa et al. 2001). They have been detected in the middle ear fluid even in the absence of bacteria during AOM (Sung et al. 1993). In nasopharyngeal swabs of symptomatic and asymptomatic AOM-prone children up to 30% detection rate of picornavirus has been seen (Pitkäranta et al. 2006). By using in situ hybridization HRV RNA has been found in 45% (up to 65% during season) of adenoid tissues from children with recurrent AOM (Rihkanen et al. 2004). HRV RNA has also been detected in the maxillary sinus during acute sinusitis (Pitkäranta et al. 2001).

6 AIMS OF THE STUDY

The general aim of these studies was to evaluate different aspects of the host immunity and pathogens in selected upper respiratory infections commonly leading to operations. Factors from both innate and adaptive immunity of the host were analyzed according to clinical findings.

Specifically, the study objectives were as follows:

1. Are there differences between the phenotypes of females and males coming for operative treatment for sinonasal diseases?

2. Is there an association between sinonasal diseases and complement upregulation?

3. Are sinonasal diseases associated with genetic deficiencies of C4 or low serum/plasma levels of immunoglobulins?

4. Does previous adenoidectomy in children modify the development of specific IgE-sensitization or allergic diseases later in life?

5. Is the clinical tonsillar disease in tonsillectomy patients associated with host immune evasion by complement regulator binding of tonsillar group A streptococci?

6. Can human rhinoviruses invade palatine tonsillar tissue and modify tonsillar disease?

7 SUBJECTS AND METHODS

7.1 Study subjects and ethical considerations

The patients for these prospective studies were recruited from the Helsinki capital area and surrounding communities belonging to the Hospital District of Helsinki and Uusimaa (**Table 6**).

Table 6. Subjects in studies. F = Female; M = Male

Group	Number	Age range	Gender M/F	Study entry	Study	Material[1]	Recruited from
Tonsillectomy patients	213	2-17	105/108	11/2002-2/2004	III, IV, V	Q, S, B, b	Department of Otorhinolaryngology Helsinki University Central Hospital Finland
Pediatric control subjects	155	7-17	78/77	4/2004	III	S, D	Elementary and junior high schools, Vihti, Finland
Chronic sinusitis patients	48	20-68	15/33	3/1996-3/2001	I	Q, S, D	Division of Infectious Diseases Helsinki University Central Hospital, Finland
Sinonasal operation patients	189	16-80	92/97	6/2003-6/2005	II	Q, S, B, b, D, CT	Department of Otorhinolaryngology Helsinki University Central Hospital, Finland
Acute sinusitis patients	50	18-83	11/39	2/2001-6/2002	I	Q, S, D	Vihti Municipal Health Center, Finland
Age and sex-matched control subjects for chronic sinusitis patients	48	20-68	15/33	5-7/2001	I	S, D	Finnish Blood Transfusion Centre
Unselected control subjects	150	18-60	49/101	2-10/2003	I, II	S, D	Vita Laboratory Ltd Helsinki, Finland

[1]Material: Q, questionnaire; S, serum; B, bacterial; D, DNA; b, biopsy; CT, computed tomography

The study protocols were approved by the ethics committees of the respective clinical units (Diary numbers 6/E5/2001, 647/E9/01, 245/E9/02), and an informed written consent was obtained from all subjects. One patient coming to sinonasal operations was found to have common variable immunodeficiency, and after an ethics committee approval the patient was informed about the disease and referred to the Immunodeficiency Unit, Division of Infectious Diseases, Department of Medicine for treatment.

7.2 Bacterial strains

In addition to bacterial culture samples from patients, five random GAS-strains from positive blood cultures (collected during 2003) and five T1M1 strains, collected and typed during years 2002-2004 at the Helsinki University Central Hospital laboratory (HUSLAB), were selected for comparison with tonsillar GAS strains.

7.3 Data collection and diagnostic definitions

Patients coming to operations (I-V) filled out a questionnaire (Appendices 1, 2 and 3) about their symptoms, history of respiratory diseases, previous sinonasal operations, the use of medication during the preceding 12 months and doctor-diagnosed allergy and asthma.

As acute sinusitis patients (study I) we recruited consecutive voluntary patients with R symptoms lasting over 7 days and fluid level or opacity in a plain sinus radiograph or purulent discharge in sinus puncture with lavage. For inclusion they had to have no previous R episodes lasting over 3 months and less than 4 yearly episodes of purulent R.

Inclusion criteria for chronic R group (sCRS) (study I) were: no clear response to sinonasal surgery other than septoplasty, to short-course antibiotics or to maximal topical medical management. Patients fulfilled Rhinosinusitis Task Force 1997 criteria **(Table 4)** (Lanza et al. 1997). Exclusion criteria for both patient groups in study I were: age less than 18 years, pregnancy, lactation, imprisonment or military service or mental retardation.

Inclusion criteria in sinonasal operation (either endoscopic sinus surgery or polypectomy) patients (SNO) (study II) were: prolonged maxillary sinusitis (over 3 months), recurrent maxillary sinusitis (more than 4 times

during one year) and/or sinonasal polyposis. Diagnosis was confirmed either with computed tomography (CT) or endoscopy. Exclusion criteria were as in study I. The diagnosis of CRS and RRS were defined as proposed by the American Academy of Otolaryngology–Head and Neck Surgery (Lanza et al. 1997). Patients were divided into different diagnosis groups as follows. Nasal polyposis was diagnosed preoperatively by nasal endoscopy, and when there were no history, symptoms or signs of sinus infections a classification nasal polyposis only (NPO) was defined. Chronic R with nasal polyposis (CRSwNP) was defined if a patient with chronic R had visible middle meatal polyposis preoperatively. Chronic R without nasal polyposis (CRSsNP) was defined when symptoms of chronic sinusitis had been present for over 12 weeks but no polyps were seen preoperatively. Recurrent R (RRS) was defined as recurrent episodes (more than 4/year) of acute bacterial sinusitis. The CRS and RRS patients were analyzed together under the term CRS. The definitions of CRSsNP and CRSwNP are not fully concordant with the later published EPOS classification, as this does not recognize recurrent R (Fokkens et al. 2007). Available CT scans were scored according to Lund-Mackay system (Lund et al. 1993). In Lund-Mackay system sinuses are individually scored in a following scale: 0=no opacification, 1=partial opacification, 2=complete opacification of each paranasal sinus and for osteomeatal unit patency: 0=open, 2=blocked. Lund-Mackay CT score ranges from 0 (no mucosal changes in any of the sinuses) to 24 (total opacification of all sinuses) points.

Inclusion criteria in tonsillectomy patients (n=213) (studies III-V) were: recurrent tonsillitis (at least 6/year or 3/year for 2 consecutive years, with at least one positive culture for GAS), or a clinical diagnosis of either chronic tonsillitis (prolonged tonsillar infection refractory to antimicrobial therapy) or tonsillar hyperplasia (enlarged tonsils with symptoms). For enrollment, children had to be without acute respiratory symptoms at the time of the operation. Not uncommonly, a child has tonsillar hyperplasia simultaneously with recurrent or chronic tonsillitis, which are all indications to operate. In study IV all indications for the operation were recorded, but the indication to operate was systematically determined: hyperplasia if no chronic or recurrent infections were noted, chronic tonsillitis if not connected with recurrent episodes of acute tonsillitis, and recurrent tonsillitis with recurrent episodes of acute exacerbations.

Pediatric controls (n=155) (study III) were randomly selected as follows: the pupils were given an ordinal number according to an alphabetical list of their family names. Thereafter, first an initial number was selected from a table of random figures and then every 10th pupil from each class level was chosen. Exclusion criterion was refusal at any stage. Only one child from each family was included.

Healthy controls (n=48) (study I) were selected from a pool of healthy blood donors (n=100) to match in age and sex with chronic R patients. These subjects had no self-reported history of R fulfilling the published criteria (Lanza et al. 1997). Exclusion criteria were the same as for patients.

Unselected controls (n=150) (studies I and II) were consecutive voluntary subjects coming for a health survey before accepting a new occupational post, again with the same exclusion criteria.

7.4 Sample collection, processing, and storage

Blood samples were drawn from each patient (studies II-V) during the operation and serum was separated in the laboratory of the Clinic of Otolaryngology, Head and Neck Surgery. In study I the patient samples were obtained from the laboratories of the Helsinki University Central Hospital (HUSLAB). From pediatric controls blood was drawn and serum separated by laboratory personnel at site. From adult controls and acute sinusitis patients the samples were taken in the corresponding laboratories. Specimens were frozen first at -20°C and transported in dry ice to the research laboratory and kept at -70°C. Genomic DNA was isolated from blood leukocytes with commercial kits (QIAamp Blood Kit, Qiagen, Austria and Puregene Kit, Gentra Systems, USA). Serum, blood and DNA samples were kept in -70°C. Details of sampling procedures are provided in the individual publications (I-V).

Tonsil samples were obtained from pediatric tonsillectomies (studies III-V). Half of the removed tonsil was snap-frozen by immersing in isopentane cooled with liquid nitrogen. After freezing the samples were kept in -70°C. The other half of the tonsil was cut into smaller pieces. From patients 51-83 (Study V) one piece was immersed in formaldehyde for in-situ examination and another was ground and cultured in blood agar plates incubated aerobically in 37°C. Standard procedures were used to determine growth of aerobic bacterial pathogens and yeasts. Streptococci were identified and the strains were stored at -70°C. The T and M serotyping of GAS strains from tonsillar tissue and blood was performed in the laboratory of the Department of Bacterial and Inflammatory Diseases, National Public Health Institute.

7.5 Analytical methods

Analytical methods used in each study are described in more detail in each article and summarized in **Table 7**.

7.5.1. Levels of complement components and immunoglobulins

Serum and plasma concentrations of C3 and C4 (Study I and II) were measured by nephelometry (Behringwerke AG, Germany). Serum CH50 (Study I) was analyzed by enzyme-linked immunosorbent assay (ELISA) technique (Quidel Corp, CA, USA). Serum immunoglobulins IgG, IgM, and IgA were measured by nephelometry using the reagents and BN ProSpec Analyzer from Dade Behring (Marburg, Germany). IgG subclasses were measured by nephelometry with Behring BNA Analyzer using PeliClass reagents (Sanquin, Amsterdam, The Netherlands).

For studies on IgE sensitization the specific IgE levels in the patients' sera were screened with the Phadiatop® (Pharmacia Diagnostics AB, Uppsala, Sweden) laboratory kit. Positive samples were further analyzed for specific IgE against the following common respiratory allergens in Finland: birch, house dust mite (*Dermatophagoides pteronyssimus*), cat, dog, horse, timothy, mugwort and mold (*Alternaria alternata*) using Pharmacia CAP System® (Pharmacia Diagnostics AB, Uppsala, Sweden) (Paganelli et al. 1998). A level of 0.35 kU/l or more of specific serum IgE was considered positive.

7.5.2. *C4A* and *C4B* typing

Allotyping of C4A and C4B was performed electrophoretically from carboxypeptidase B (Roche Diagnostics Gmbh, Mannheim, Germany) and neuraminidase (Sigma-Aldrich Chemie Gmbh, Type IV, Steinheim, Germany) treated serum samples followed by immunofixation with polyclonal anti-C4 antibody (DiaSorin Inc., Stillwater, MN, USA) with the standard procedure (Marcus D 1996). C4A and C4B allotypes were run to specific positions on the gel in relation to the standards. The presence of ≤ 1 C4A or C4B variants were defined as nulls.

The copy number variations of *C4A* and *C4B* genes were analyzed by isotype-specific genomic real-time polymerase chain reaction (RT-PCR) (Seppänen et al. 2006). *C4A* pseudogene caused by a 2-base pair insertion in exon 29 (codon1213) was analyzed by sequence specific RT-PCR (Barba et al. 1994). We used unlabeled primers with SYBR Green QPCR (Stratagene, Cedar Creek, Texas, USA) or Absolute QPCR SYBR GREEN MIX (Abgene, Epsom, UK) according to the manufacturers' instructions with minor modifications. The presence of one or less functional *C4A* or *C4B* gene defined a deficiency.

7.5.3. GAS typing and binding of C regulators

Factor H (Calbiochem) and C4BP (a gift from Dr.Anna Blom, University of Lund, Sweden) proteins were radiolabeled with ^{125}I using the Iodogen method for use in the binding assays (Salacinski et al. 1981). GAS strains were grown overnight in Todd-Hewitt broth, washed three times with veronal-buffered saline, containing 0.1% gelatin (GVB). C4BP binding assays were done in GVB and FH binding assays in 1/3 GVB. The bacterial concentration was adjusted to 9.5 x 10^8/ ml. A total of 20 µl portions of the suspension (containing 1.9x10^7 bacteria) were mixed with ^{125}I -labeled FH or ^{125}I -labeled C4BP and incubated for 30 min at 37°C. Bacteria-bound and free radioactive proteins were separated by centrifuging the samples through a 250 µl column of 20% sucrose in GVB (C4BP) or 1/3 GVB (FH). The bottoms of the tubes were cut out and radioactivities in both the supernatants and the pellets were measured using a gammacounter. Binding is expressed as percentage of the radioactivity in pellet vs total radioactivity (pellet+supernatant). All experiments were performed in triplicate or quadruplicate.

T serotypes and *emm* types were determined in GAS strains cultured from tonsils or blood. T serotyping was performed using five polyvalent and 21 monovalent anti-T-agglutination sera (Sevac Ltd., Czech Republic) (Moody et al. 1965) . M-protein type was determined with *emm* sequence typing using primers MF1 (forward) and MR1 (reverse): for *emm*-PCR (Siljander et al. 2006). PCR products were purified with the QIAquick PCR purification Kit (Qiagen) as described by the manufacturer. The *emm*-sequencing reaction was performed with the primer MF1 and BigDye chemistry (Applied Biosystems) as described by the manufacturer, and analyzed with an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The sequence data was analyzed by BLAST search against *S. pyogenes emm* sequence database at Centers for Disease Control and

Prevention (CDC). The *emm*-types were defined as having 95% sequence identity with the exact 150 base type-specific sequences in the database as described at the CDC website.

7.5.4. Detection of rhinoviruses

Preparation of the rhinovirus-14 probe and the in-situ hybrization (ISH) protocol were based on published procedures (Pitkäranta et al. 2001) The result in ISH was considered positive when the hybridization signal was visible as cytoplasmic reactivity in more than 10% of either epithelial or lymphoid cells. The specificity of the ISH signal was verified by *in vitro* infection of HeLa cells with rhinovirus-14. Uninfected HeLa cells were negative both by the antisense and by the sense probe. It should be noted that although the genomic region exploited was designed to be HRV specific we cannot totally exclude partial cross-reactivity with some human enterovirus (HEV) strains. The results were evaluated in a blind manner from coded slides and the evaluators had no information about the PCR or culture results, or the clinical background of the patient.

We used two different reverse transcription polymerase chain reaction (RT-PCR) methods for HRV/HEVRNA detection. DNA amplification was done in parallel with two different methods. In the conventional PCR-hybridization assay (PCR-HYB) the polymerase reaction was carried out on microtiter plates as described earlier (Blomqvist et al. 1999). The other method used was a single-tube nested PCR, a hanging drop version (nested PCR) modified from that described by Walsh and Ratcliff (Walsh et al. 2001; Ratcliff et al. 2002).

Table 7. Laboratory methods used in the studies.

Sample	Method	Described in detail in study
Serum	Allotyping of C4A and C4B proteins by electrophoresis and immunofixation	I
Serum/plasma	C3, C4, IgM, IgA, IgG, IgG1, IgG2, IgG3, IgG4 concentrations by nephelometry	I, II,
Serum	Specific IgE for respiratory allergens by Phadiatop®	II, III
Isolated streptococci	Radiolabeled protein binding assays	IV
Isolated streptococci	Typing of T and M surface proteins by serology, PCR and sequencing	IV
Genomic DNA	Quantitative isotype-specific real time PCR amplification of *C4A* and *C4B*	I, II
Tonsillar tissue	HRV-ISH	V
Tonsillar tissue	RT-PCR for HRV/HEV-RNA	V

7.6 Statistical analyses

In studies I, II and III, χ^2 (Chi-square) and Fisher's exact two-tailed tests were used for analysis of differences in categorical data between groups, as appropriate. Mann-Whitney U-test was used for comparing continuous data in studies IV and V. Forward stepwise logistic regression analysis was used in studies I, IV and V to identify the influence of different predictors to clinical symptoms and forms of disease. In study I comparisons between all groups in continuous variables were performed by nonparametric analysis of variance (Kruskal–Wallis test, Jonkcheere–Terpstra test for ordinal groups), as most variables had non-normal distribution in at least one group. If the variance analysis showed significant differences between groups, the two-tailed Student's t-test with Bonferroni correction was employed. In study I the analysis of C4Q0 frequencies between study groups, odds ratios (OR) and 95% confidence interval (CI) were calculated using EpiInfo version 6, and tested using logistic regression analysis.

Statistical analyses were performed using SPSS 12.0.1. for Windows (studies I and III) and SPSS 16 for Mac (study II) (SPSS Inc., Chicago, IL, USA) and Statsdirect (studies II, III, IV and V) statistical softwares (version 2.3.5, Statsdirect, Cheshire, UK). Missing values were excluded from the analyses.

8 RESULTS

8.1 Clinical and laboratory findings in adult patients with sinonasal diseases

8.1.1 Overall clinical observations between adult patient groups (studies I and II)

To analyze clinical features in various sinonasal diseases a large data pool from 287 adult patients with different clinical forms of the diseases was collected. From this data, comparisons on the special features and sex differences between each studied group could be made. Clinical characteristics of the different patient groups with sinonasal diseases are shown in **Table 8a and 8b.**

CRSsNP patients were younger than all other SNO patients [($p<0.001$ in females (F) and males (M)]. In females CRSsNP patients and in males ARS patients were the youngest. The median ages of both female and male patients with polyposis were higher than those of all other patient groups.

Positive smoking history was common in SNO patients (57% F; 60% M) and ARS patients (F 54%, M 73%), but uncommon in severe sinusitis patients (F 30%, M 20%), ($p=0.008$ F, $p=0.005$ M). Current smokers were most commonly found in ARS (F 36%, M 36%) and NPO (F 33%, M 31%) patients.

The frequency of allergic symptoms varied largely between different sinonasal disease groups but only little between females and males. Reported allergic symptoms were very common (>50%) in all SNO patients, but uncommon in female ARS patients (15%) ($p<0.001$). Non-steroidal anti-inflammatory drug (NSAID) intolerance was more common in patients with NPO (F 25%, M 31%) and in patients with sCRS (F 27%, M 33%) than in patients with ARS (F 0%, M 0%) ($p=0.010$ F, $p=0.04$ M; $p<0.001$ F, $p=0.052$ M, respectively) or CRSsNP (F 0%, M 3%) ($p=0.003$ F, $p=0.004$ M; $p<0.0001$ F, $p=0.011$ M, respectively). Bronchial asthma in females was most common in CRSwNP patients (65%) and least common in ARS (10%) ($p<0.001$) and CRSsNP (26%) ($p=0.003$) patients. In males bronchial asthma was most common in severe sinusitis patients (60%) and least common in acute (0%) ($p<0.001$) and CRSNP (10%) ($p<0.001$) patients. Specific IgE to respiratory allergens in SNO patients was more common in males than females (F 28%, M 42%) ($p= 0.047$) .

Table 8a and 8b. Clinical findings and comorbidities in adult patients with sinonasal diseases. I=patients from study I, II=patients from study II.

8a. Females

	Sinonasal operation patients (II)				Severe (I)	Acute (I)	Global p-value
	All[1] n (%)	CRSsNP[1] n (%)	CRSwNP[1] n (%)	NPO[1] n (%)	sCRS[1] n (%)	ARS[1] n (%)	
Number of patients	97	65	20	12	33	39	
Age median (range)	40 (16-80)	35 (16-66)	53 (28-80)	53 (33-64)	41 (20-68)	46 (18-83)	<0.001[2]
Ever smoked	55 (57)	33 (51)	13 (65)	9 (75)	10 (30)	21 (54)	0.039[3]
Current smoker	19 (20)	13 (20)	2 (10)	4 (33)	2 (6)	14 (36)	0.011[3]
Allergic symptoms	62 (64)	40 (62)	15 (75)	7 (58)	24 (73)	6 (15)	<0.001[3]
NSAID-intolerance	7 (7)	0 (0)	4 (20)	3 (25)	9 (27)	0	<0.001[4]
Specific IgE to respiratory allergens	27 (28)	20 (31)	4 (20)	3 (25)	[5]	[5]	0.626[3]
Exposure to nasal irritants	26 (26)	16 (25)	7 (35)	3 (25)	10 (30)	8 (21)	0.605[2]
Nasal polyposis	32 (33)	[6]	[6]	[6]	18 (55)	3 (8)	<0.001[3]
Deviated nasal septum	14 (14)	10 (15)	1 (5)	3 (25)	5 (15)	1 (3)	0.116[4]
Nasal fractures	3 (3)	2 (3)	0 (0)	1 (8)	0 (0)	1 (3)	0.480[3]
Nasal steroid use	72 (74)	47 (72)	15 (75)	10 (83)	[5]	2 (5)	<0.001[4]
Upper molar infections	21 (22)	15 (23)	4 (20)	2 (17)	1 (3)	0 (0)	0.0037[4]
Bronchial asthma	36 (37)	17 (26)	13 (65)	6 (50)	18 (55)	4 (10)	<0.001[3]
CT score median (range)	11 (0-24)	9 (0-24)	16 (4-24)	16 (13-24)	7 (0-22)	[5]	<0.001[2]

[1] All = all patients coming to sinonasal operation, CRSsNP = chronic rhinosinusitis without nasal polyposis, CRSwNP = chronic rhinosinusitis with nasal polyposis, NPO = nasal polyposis only, sCRS = severe chronic rhinosinusitis, ARS = acute/intermittent (presumed bacterial) rhinosinusitis

[2] Mann-Whitney U-test

[3] Chi-square test

[4] Fisher's exact test

[5] not recorded

[6] part of group definition

The extent of both visual and radiological mucosal changes in nose and paranasal sinuses was associated to sex and to the chronicity of the disease. Nasal polyposis was more common in all severe sinusitis (F 55%, M 73%) patients than in acute sinusitis (F 8%, M 0%) patients ($p<0.001$ for both). It was also more common in male SNO patients than female SNO patients (F 33%, M 67%, $p<0.001$). CT scores, indicating the extent of the mucosal disease, were lowest in female patients with severe CRS (CT score median=7) and CRSsNP (CT score median=9). Only in CRSwNP the female patients had higher CT scores (F 16, M 14) than males, all other groups had lower scores in females than males.

8b. Males

	Sinonasal operation patients (II)				Severe (I)	Acute (I)	Global p-value
	All[1] n (%)	CRSsNP[1] n (%)	CRSwNP[1] n (%)	NPO[1] n (%)	sCRS[1] n (%)	ARS[1] n (%)	
Number of patients	92	30	26	36	15	11	
Age median (range)	46 (18-75)	37 (18-62)	46 (28-73)	53 (23-75)	38 (29-58)	31 (25-64)	<0.001[2]
Ever smoked	55 (60)	19 (63)	14 (54)	22 (61)	3 (20)	8 (73)	0.037[3]
Current smoker	23 (25)	5 (17)	6 (23)	11 (31)	0 (0)	4 (36)	0.108[3]
Allergic symptoms	48 (52)	16 (53)	15 (58)	27 (75)	10 (66)	4 (36)	0.145[3]
NSAID-intolerance	15 (16)	1 (3)	3 (12)	11 (31)	5 (33)	0 (0)	0.005[4]
Specific IgE to respiratory allergens	39 (42)	10 (33)	11 (42)	18 (50)	[5]	[5]	0.394[3]
Exposure to nasal irritants	28 (30)	9 (30)	10 (38)	9 (25)	5 (33)	3 (27)	0.843[4]
Nasal polyposis	62 (67)	[6]	[6]	[6]	11 (73)	0 (0)	<0.001[4]
Deviated nasal septum	29 (32)	6 (20)	9 (35)	14 (39)	5 (33)	1 (9)	0.246[3]
Nasal fractures	10 (11)	5 (17)	3 (12)	2 (6)	0 (0)	2 (18)	0.287[4]
Nasal steroid use	49 (53)	17 (56)	19 (73)	23 (64)	[4]	1 (9)	0.003[3]
Upper molar infections	21 (23)	8 (19)	4 (15)	9 (25)	0 (0)	2 (18)	0.231[3]
Bronchial asthma	34 (37)	3 (10)	12 (46)	19 (53)	9 (60)	0 (0)	<0.001[3]
CT score median (range)	14 (1-24)	10 (1-18)	14 (10-24)	23 (2-24)	14 (0-24)	[5]	<0.001[2]

[1] All = all patients coming to sinonasal operation, CRSsNP = chronic rhinosinusitis without nasal polyposis, CRSwNP = chronic rhinosinusitis with nasal polyposis, NPO = nasal polyposis only, sCRS = severe chronic rhinosinusitis, ARS = acute/intermittent (presumed bacterial) rhinosinusitis
[2]Mann-Whitney U-test
[3] Chi-square test
[4] Fisher's exact test
[5] not recorded
[6] part of group definition

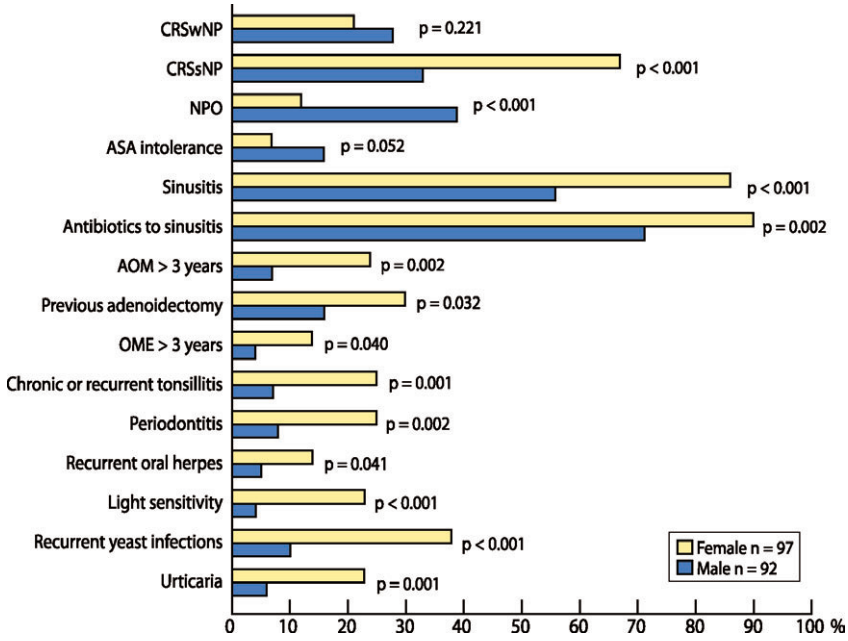
8.1.2 Sex differences in respiratory and mucosal infections (study II)

Although the inclusion criteria for females and males coming to sinonasal operations were the same, they presented with remarkably different pattern of past and present infectious diseases. The differences between female and male SNO patients are shown in **Figure 5**.

As an indication for operation females had more often CRSsNP (F 67% vs M 33%, $p<0.001$) and males NPO (M 39% vs F 12%, $p<0.001$). No differences were seen in CRSwNP (F 21% vs M 28%). Females reported more sinusitis (F 86% vs. M 56%, $p<0.001$) and use of antibiotics for sinusitis (F 90% vs M 71%, $p=0.002$). There was also a significant difference in the number of courses of antibiotics prescribed during the preceding year (mean F 4.1 vs. M 2.3, $P<0.001$).

Acute otitis media (AOM) at over 3 years of age was reported by 24% of females and by 7% of males ($p=0.002$), and otitis media with effusion (OME) by 14% of females and by 4% of males ($p=0.040$). Adenoidectomy had been done to 30% of females and 16% of males ($p=0.032$). Chronic or recurrent tonsillitis was reported by 25% of females and by 7% of males ($p=0.001$). 25% of females and 8% of males reported of being prone to develop periodontitis ($p=0.002$) and 14% of females and 5% of males of having recurrent oral herpes (8 times per year or 4 times per 6 months) ($p=0.041$).

Figure 5. Differences in respiratory infections between females and males coming to sinonasal operations.



CRSwNP = chronic rhinosinusitis with nasal polyposis, CRSsNP = chronic rhinosinusitis without nasal polyposis, NPO = nasal polyposis only, ASA = aspirin (acetylsalicylic acid), AOM=acute otitis media, OME=otitis media with effusion

8.1.3. Complement C3, C4 and CH50 levels in rhinosinusitis patients

Complement component levels are able to indicate ongoing inflammation. Serum C3 and C4 levels and complement classical pathway activity (CH50) were found to be elevated in male and female patients with ARS, when compared to controls ($p<0.001$ for C3, C4 and CH50) or to all other groups ($p<0.001$ for C3, C4). C3 and C4 levels were elevated only in males but not in females when SNO patients were compared with controls ($p<0.001$ and 0.096 respectively). **Table 9a and 9b**.

8.1.4 Plasma immunoglobulin levels in rhinosinusitis patients

As females and males have been suggested to mount different adaptive immunity responses, total plasma Ig levels were studied to find out whether differences in antibody levels could be related to the different phenotypes of sinonasal disease between females and males. Differences in mean plasma values of Igs and the number of values below reference (VBR; mean-2SD) in females and males are shown in **Table 9a and 9b**.

When all SNO patients were analyzed together against all controls (Study II) lower levels of IgG ($p=0.010$), IgG1 ($p<0.001$), IgG3 ($p=0.015$), and more values below reference (VBR) for IgG ($p=0.004$), IgG1 ($p<0.001$), IgG3 ($p=0.003$) were seen. SNO patients had higher levels of IgA ($p=0.003$) and IgG2 ($p=0.004$) than controls. In all female SNO patients, lower levels of IgG ($p=0.02$), IgG1 ($p<0.001$) and IgG3 ($p=0.009$) were found when compared to female controls. This phenomenon was most pronounced in female CRSwNP patients. Also higher levels of IgG2 ($p=0.034$) were seen in all female SNO patients when compared to female controls. When VBR were compared, female SNO patients had more VBR in IgG1 ($p<0.001$) and male SNO patients more VBR in IgG3 levels ($p=0.014$) than their sex-matched controls. IgG4 levels in male CRSsNP patients were lower than in male NPO patients. In female patients with ARS, levels of total IgG ($p<0.001$), and of subclasses IgG1 ($p=0.004$) and IgG3 ($p=0.008$) were lower than in female controls. In males this difference was not significant.

Higher levels of IgA were seen in females with NPO ($p=0.02$) and lower levels of IgM in females with CRSwNP ($p=0.05$) when compared with female controls.

Table 9a and 9b. Complement C3 and C4 and immunoglobulin levels in patients with sinonasal disease. Mean serum or plasma values in patients and controls are shown. Values in parenthesis indicate the number of values below reference. I= patients from study I, II= patients from study II, n= number of patients within group.

9a. Females

	Sinonasal operation patients (II)				(I)	(I,II)	Global p-value[3]
	All	CRSsNP	CRSwNP	NPO	ARS	Controls	
Parameters[1]	n=97	n=65	n=20	n=12	n=39	n=101	
C3	1.03 (7)	1.02 (2)	1.01 (3)	1.11 (2)	1.27 (0)	1.03 (5)	<0.001 0.238#
C4	0.19 (9)	0.21 (6)	0.18 (2)	0.20 (1)	0.25 (1)	0.19 (8)	<0.001 0.759#
CH50[4]	[5]	[5]	[5]	[5]	177 (0)	119 (2)	<0.001&
IgA	2.01 (1)	1.95 (0)	1.96 (1)	2.40 (0)	1.83 (0)	1.80 (1)	0.223 0.279#
IgM	1.29 (4)	1.43 (2)	1.01 (2)	0.98 (0)	1.16 (1)	1.31 (4)	0.007 0.616§
IgG	10.6 (5)	10.9 (1)	9.8 (3)	10.2 (1)	9.34 (3)	11.3 (0)	<0.001 0.006#
IgG1	5.97 (20)	6.08** (11)	5.63 (7)	5.97 (2)	6.11 (8)	6.95 (4)	<0.001 <0.00§
IgG2	3.54 (3)	3.73** (0)	2.94* (2)	3.52 (1)	3.18 (5)	3.19 (2)	0.034 0.004#
IgG3	0.30 (24)	0.31 (11)	0.28 (8)	0.27 (5)	0.29 (13)	0.35 (15)	0.015 0.017§
IgG4	0.56 (3)	0.53 (1)	0.58 (1)	0.71 (1)	0.51 (1)	0.57 (6)	0.867 0.158#

[1] Except for CH50 (IU/ml) all values are shown as g/l.

[2] All = all patients coming to sinonasal operation, CRSsNP = chronic rhinosinusitis without nasal polyposis, CRSwNP = chronic rhinosinusitis with nasal polyposis, NPO = nasal polyposis only, ARS = acute/intermittent (presumed bacterial) rhinosinusitis

[3] Comparisons between mean level values were made with nonparametric ANOVA (Kruskall-Wallis) and those between numbers of values below reference with §Chi-square and #Fisher's exact text. & When appropriate, mean values were compared with Student's t-test with Bonferroni correction. * $p<0.05$, ** $p<0.01$ as compared with controls

[4] Values ≥200 IU/ml counted as 200

[5] Not recorded

9b. Males

	Sinonasal operation patients (II)				(I)	(I,II)	Global p-value[3]
	All[2]	CRSsNP[2]	CRSwNP[2]	NPO[2]	ARS[2]	Controls	
Parameters[1]	n=92	n=30	n=26	n=36	n=11	n=49	
C3	1.17 (5)	1.18 (1)	1.13 (2)	1.20 (2)	**1.38** (0)	1.04 (0)	<0.001 0.287[#]
C4	0.24 (3)	0.23 (1)	0.22 (1)	0.26 (1)	**0.30** (0)	0.20 (1)	<0.001 >0.999[#]
CH50[4]	[5]	[5]	[5]	[5]	183 (0)	109 (2)	<0.001[&]
IgA	2.35 (6)	2.22 (2)	2.45 (2)	2.39 (2)	2.10 (1)	2.08 (4)	0.592 0.504[#]
IgM	0.97 (5)	0.92 (2)	0.97 (1)	1.01 (2)	1.01 (0)	1.03 (3)	0.744 0.783[#]
IgG	10.44 (5)	10.0 (3)	10.5 (2)	10.8 (0)	9.52 (0)	10.9 (0)	0.405 0.033[#]
IgG1	6.00 (21)	5.76 (8)	5.99 (8)	6.21 (5)	6.40 (0)	6.49 (6)	0.298 0.016[§]
IgG2	3.50 (4)	3.30 (2)	3.50 (1)	3.67 (1)	3.00 (1)	3.06 (1)	0.132 0.612[#]
IgG3	0.30 (26)	0.30 (11)	0.31 (6)	0.30 (9)	0.26 (4)	0.33 (5)	0.488 0.064[§]
IgG4	0.80 (2)	**0.53*** (2)	0.99 (0)	**0.88*** (0)	0.97 (1)	0.69 (2)	0.010 0.307[#]

[1] Except for CH50 (IU/ml) all values are shown as g/l.

[2] All = all patients coming to sinonasal operation, CRSsNP = chronic rhinosinusitis without nasal polyposis, CRSwNP = chronic rhinosinusitis with nasal polyposis, NPO = nasal polyposis only, ARS = acute/intermittent (presumed bacterial) rhinosinusitis

[3] Comparisons between mean level values were made with nonparametric ANOVA (Kruskall-Wallis) and those between numbers of values below reference with [§]Chi-square and [#]Fisher's exact text. [&] When appropriate, mean values were compared with Student's t-test with Bonferroni correction. * $p<0.05$, ** $p<0.01$ as compared with controls

[4] Values ≥200 IU/ml counted as 200

[5] Not recorded

8.1.5 Numbers of functional *C4A* and *C4B* genes in rhinosinusitis patients

Complement C4 function is crucial for one of the strongest innate immunity defense mechanisms, the complement system. Therefore, it was studied if missing *C4A* or *C4B* genes could affect the sinonasal disease susceptibility in females or males. The frequencies of *C4A* and *C4B* deficiencies (a lack of at least one *C4* gene) in patients and controls are shown in **Table 10a and 10b**.

An increased number of *C4A* deficiencies was seen in females with sCRS as compared with female controls (36 % vs. 17%, p=0.048). In males, this was not significant due to smaller groups (33% vs. 14% p=0.13). Total *C4A* deficiency was more common in males with sCRS as compared with male controls (13% vs 0%, p=0.052), but not significant in females (5% vs. 2%, p=0.096). No differences in partial or total *C4A* deficiencies were seen between SNO or ARS patients and controls.

An increased number of *C4B* deficiencies in female SNO patients as compared to female controls (62% vs. 38%; p=0.001) was found. No such difference was seen in male SNO patients (41% vs. 47% in controls). *C4B* deficiencies in female SNO patients were also more common than in male SNO patients (p=0.005). The number of total *C4B* deficiencies was also high in female SNO patients (18%, controls 9%), but the difference was not significant (p=0.09).

When divided by clinical diagnosis, females with CRSwNP had a significantly higher incidence of *C4B* deficiency (74%) than female controls (38%, p<0.001). A complete *C4B* deficiency (two *C4B* null alleles) was found in 37% (7/19) of female patients with CRSwNP. This was also significantly higher than in controls (9%; p=0.003). Significance between female and male CRSwNP patients was p=0.001 for partial and p<0.0001 for total deficiency. The high incidence of *C4B* deficiency was found in CRSsNP patients (56/93=60%, p=0.003) as compared with controls (61/150=41%) (p=0.003). When divided by sex, *C4B* deficiencies had the same incidence in females (60%; n=63) and males (60%; n=30), but the difference to gender matched controls (38% F, 47% M) was only significant in females (p=0.005 F, p=0.26 M).

In female patients coming to sinonasal operations the likelihood of having *C4B* deficiencies was higher than in female controls, OR 2.75 (95% CI 1.48-4.91) p=0.001. In female CRSsNP patients compared with female controls, OR for *C4B* deficiency was 2.52 (95% CI 1.26-5.06) p=0.006. In female CRSwNP patients compared with female controls, OR for *C4B* de-

ficiency was 4.64 (95% CI 1.41-17.6) p=0.005 and for total *C4B* deficiency 5.96 (95% CI 1.55-21.7) p=0.004.

Male patients with CRSwNP had the lowest incidence (11%) of *C4B* deficiency of all male or female patient groups or controls and none had complete *C4B* deficiency. This difference was highly significant (p=0.002 to male controls, p=0.0003 to other male SNO patients).

Since the male control group was relatively small, the analyses were repeated with a larger control group by including gender- and age-matched healthy controls from blood donors in study I with available data. The prevalence of *C4B* deficiency was 37% in 134 female controls and 45% in 64 male controls. The prevalence of total *C4B* deficiency in female and male controls was 9% and 13%, respectively. Statistical results remained essentially unchanged (data not shown).

Table 10a and 10b. Frequencies of *C4* deficiencies in female (A) and male (B) patients in different clinical disease groups as compared to controls. I=patients from study I, II=patients from study II.

10a. Females

	Sinonasal operation patients (II)				(I)	(I)	(I,II)	Global p-value
	All[a]	CRSsNP[a]	CRSwNP[a]	NPO[a]	sCRS[a]	ARS[a]	Controls	
***C4* gene numbers**	n=93	n=63	n=19	n=11	n=33	n=39	n=101	
C4A<2	14 (15)	8 (13)	3 (16)	3 (10)	12 (36)	4 (10)	18 (17)	0.051[C]
C4A=0	1 (1)	0 (0)	0 (0)	1 (10)	3 (10)	2 (5)	2 (2)	0.057[F]
C4B<2	58 (62)	38 (60)[1]	14 (74)[2]	6 (55)	15 (45)	18 (46)	38 (38)	0.019[C]
C4B=0	17 (18)	8 (13)	7 (37)[3]	2 (18)	1 (3)	2 (5)	9 (9)	0.008[F]

[a] All = all patients coming to sinonasal operation, CRSsNP = chronic rhinosinusitis without nasal polyposis, CRSwNP = chronic rhinosinusitis with nasal polyposis, NPO = nasal polyposis only, sCRS = severe chronic rhinosinusitis, ARS = acute/intermittent (presumed bacterial) rhinosinusitis
C4 deficiencies n (% within group) Chi-square [C] and Fisher's exact test [F] when appropriate, [1] p=0.023, [2] p=0.019, [3] p=0.003

10b. Males

	Sinonasal operation patients (II)				(I)	(I)	(I,II)	Global p-value
	All[a]	CRSsNP[a]	CRSwNP[a]	NPO[a]	sCRS[a]	ARS[a]	Controls	
***C4* gene numbers**	n=91	n=30	n=26	n=35	n=15	n=11	n=49	
C4A<2	15 (16)	3 (10)	5 (19)	7 (20)	5 (33)	0	7 (14)	0.253[F]
C4A=0	1 (1)	0 (0)	1 (4)	0 (0)	2 (13)	0	0 (0)	0.02[F]
C4B<2	37 (41)	18 (60)	**3 (11)**[1]	16 (46)	8 (53)	6 (55)	23 (47)	0.009[C]
C4B=0	4 (4)	1 (3)	0 (0)	3 (8)	2 (13)	0	6 (12)	0.288[F]

[a] All = all patients coming to sinonasal operation, CRSsNP = chronic rhinosinusitis without nasal polyposis, CRSwNP = chronic rhinosinusitis with nasal polyposis, NPO = nasal polyposis only, sCRS = severe chronic rhinosinusitis, ARS = acute/intermittent (presumed bacterial) rhinosinusitis
C4 deficiencies n (% within group) Chi-square [C] and Fisher's exact test [F] when appropriate, [1] p=0.002

8.2 Clinical and laboratory findings in pediatric patients with tonsillar diseases

8.2.1 Clinical observations in pediatric patients

Tonsillectomies are among the most frequent pediatric operations. Sex differences are also seen in the incidence and clinical manifestations of tonsillar diseases that are most common before adulthood. In this study factors influencing the susceptibility to tonsillar diseases and consequences of previous adenoid surgery to the development of allergies later on were investigated.

For these studies, we recruited 213 children (aged 2-16.9 years), coming to scheduled tonsillectomy. The median age of the patients at the time of tonsillectomy was 7.6 years. The indication for tonsillectomy was tonsillar hypertrophy in 89%, recurrent tonsillitis in 27% and chronic tonsillitis in 26% of children. There were, on the average, 1.4 indications per operation. The primary indication (study IV) was tonsillar hypertrophy in 60%, recurrent tonsillitis in 20% and chronic tonsillitis in 20% of the children. The indication for tonsillectomy was strongly age-dependent. In children under 7 years of age, tonsillar hypertrophy was the most common (80%) primary operation indication, recurrent tonsillitis (8%) and chronic tonsillitis (10%) were less common. In children over 7 years of age tonsillar hypertrophy (40%) was still the main primary indication followed by recurrent tonsillitis (31%) and chronic tonsillitis (29%). No differences were found in the profiles of observed microbial flora (group A, B, C and G betahemolytic streptococci, *Staphylococcus aureus*, yeasts) in the tonsils of children under or over 7 years of age (data not shown). **Table 11** shows clinical data of tonsillectomized children divided by age and sex. Snoring (p=0.002) and sleep apnea (p=0.009) were more common in boys than girls over 7 years of age.

Table 11. Main clinical characteristics between girls and boys of different age groups coming to tonsillectomy[1]. Statistically significant results bolded.

	Age <7				Age ≥7			
	All	**Female**	**Male**	**p-value**	**All**	**Female**	**Male**	**p-value**
Number of patients (%)	98	44 (45)	54 (55)		115	64 (56)	51 (44)	
Age median	4.5	4.4	4.6	0.988[2]	11.7	**12.7**	**10.7**	**0.004[2]**
Recurrent URTI[4]	39 (40)	17 (39)	22 (41)	>0.999[3]	64 (55)	36 (55)	28 (55)	>0.999[3]
Recurrent OME[5]	90 (92)	40 (91)	50 (93)	>0.999[3]	83 (72)	42 (65)	41 (80)	0.096[3]
Recurrent sinusitis	10 (10)	6 (14)	4 (7)	0.337[3]	37 (32)	18 (28)	19 (37)	0.321[3]
Chronic rhinitis	36 (37)	15 (34)	21 (39)	0.677[3]	33 (28)	15 (23)	18 (35)	0.213[3]
Smoking at home	34 (35)	12 (27)	22 (41)	0.203[3]	38 (33)	20 (31)	18 (35)	0.693[3]
Previous adenoidectomy	43 (44)	15 (34)	28 (52)	0.102[3]	57 (49)	29 (45)	28 (55)	0.351[3]
Snoring	93 (95)	40 (91)	53 (98)	0.170[3]	80 (69)	**37 (57)**	**43 (84)**	**0.002[3]**
Sleep apnea	47 (48)	21 (48)	26 (48)	>0.999[3]	23 (20)	**7 (11)**	**16 (31)**	**0.009[3]**
Chronic tonsillitis	10 (10)	3 (7)	7 (13)	0.504[3]	46 (40)	30 (46)	16 (31)	0.125[3]
Recurrent tonsillitis	13 (13)	6 (14)	7 (13)	>0.999[3]	45 (39)	29 (45)	16 (31)	0.178[3]
Tonsillar hyperplasia	95 (97)	43 (98)	52 (96)	>0.999[3]	95 (82)	51 (78)	44 (86)	0.456[3]

[1] Results are shown as n (%)
[2] Mann-Whitney U test
[3] Chi-square test
[4] URTI= Upper respiratory tract infections
[5] OME= Otitis media with effusion

8.2.2 Allergic diseases and previous adenoidectomy in tonsillectomy patients

Adenotonsillar tissue belongs to the upper respiratory tract immune system. It has an important role in the development of allergic sensitization, since respiratory and food allergens have to pass the Waldeyer's ring to enter the body. In this work, the aim was to explore the role of allergy as a comorbid condition for adenotonsillectomy. The other point of interest was to see if previously performed adenoidectomy would predispose to the development of allergic diseases or IgE-mediated sensitization to respiratory allergens.

Table 12 shows clinical characteristics in tonsillectomy patients divided into two groups according to preceding adenoidectomy (n=100) or the lack of it (n=113). The presence of specific serum IgE for common Finnish respiratory allergens was screened from the sera of all patients. The patient groups were compared with each other. Patients over 7 years of age (n=155) were also compared to a group of randomly selected school children (n=155) of the same age.

The results showed that recurrent otitis media ($p<0.001$), sinusitis ($p=0.001$), recurrent sinusitis ($p=0.007$) and rhinitis ($p=0.011$) were more common in the adenoidectomized children.

Comparisons of the prevalence of allergy, asthma and the presence of allergen-specific IgE in different age groups according to previous adenoidectomy are shown in **Figure 6** and different clinical allergic diseases divided by age and sex in **Figure 7**. In children under the age of seven, any allergy ($p=0.007$), non-antibiotic allergy diagnosed by a doctor ($p=0.015$) and asthma ($p=0.015$) were more commonly seen if adenoidectomy had been done previously. Between ages 7 and 11 neither allergy nor asthma was associated with previous adenoidectomy. In children between 12 and 17 years of age, only antibiotic allergy was more common if adenoidectomy had been done previously. In children under 7 years of age, doctor-diagnosed non-antibiotic allergy was significantly associated with reported recurrent upper respiratory tract infections OR 3.7 (95% CI 1.0-13.6) $p=0.046$ and previous adenoidectomy OR 4.8 (95%CI 1.2-19.3) $p=0.027$. Previous adenoidectomy did not associate with the appearance of respiratory allergen-specific IgE later in life.

Of the 175 patients without a diagnosis of allergy to respiratory allergens, 17 (10%) had specific IgE against at least one of the tested allergens and 8 (5%) were multisensitized. Out of 24 asthmatic patients, 17 (71%) had been diagnosed with allergy: 16 to respiratory allergens, six to foods and

Table 12. Clinical characteristics of pediatric patients coming to tonsillectomy (study III) divided into groups by previous adenoidectomy. Statistically significant results bolded. With permission from Rhinology.

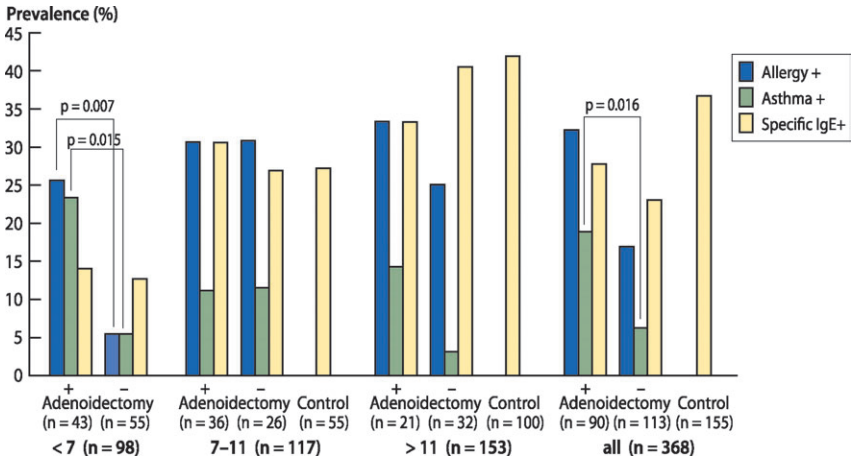
	All tonsillectomised children n (%)	Previous adenoidectomy n (%)	No previous adenoidectomy n (%)	p-value[4]
No of patients	213	100	113	
Median age (range)[1]	7.6 (2.0-16.9)	8.1 (2.0-16.6)	7.1 (2.0-16.9)	0.825[5]
Males	105 (49)	56 (56)	49 (43)	0.065[6]
Recurrent URTI[2]	103 (48)	55 (55)	48 (42)	0.068[6]
Recurrent OME (>3)[3]	122 (57)	**75 (75)**	**47 (42)**	**<0.001[6]**
Sinusitis[2]	47 (22)	**32 (32)**	**15 (13)**	**0.001[6]**
Recurrent sinusitis (>3)[3]	10 (5)	**9 (9)**	**1 (1)**	**0.007[6]**
Chronic rhinitis[2]	67 (31)	**40 (40)**	**27 (24)**	**0.011[6]**
Snoring[2]	172 (81)	82 (82)	90 (80)	0.664[6]
Smoking at home	71 (33)	38 (38)	33 (29)	0.174[6]
Day care	78 (37)	36 (36)	42 (37)	0.859[6]

[1] Years
[2] Considered by guardian
[3] >3 episodes of otitis/sinusitis verified by doctor
[4] p-values between adenoidectomy+ and adenoidectomy- groups
[5] Mann-Whitney U test
[6] Chi-square test
URTI= Upper respiratory tract infections
OME= Otitis media with effusion

two to antibiotics. Specific IgE was found in 13 (54%) of asthmatics and 11 (45%) of them were multisensitized. Twelve (75%) of 16 asthmatics previously diagnosed with allergy to respiratory allergens had specific IgE against the tested respiratory allergens, 11 (69%) against several of them.

In female patients under 7 years of age, specific IgE to respiratory allergens was often seen (20% vs. 7 % in male patients; p=0.058) (**Figure 8**). In tonsillectomized boys over the age of 7, specific IgE (p=0.040), doctor-diagnosed allergy (p=0.014), allergy to dusts (p=0.004) and doctor-diagnosed non-antibiotic allergy (p=0.015) were found more often than in girls (**Figures 7 and 8**). Although snoring and sleep apnea acknowledged by parents was more common in boys over 7 years than in girls of the same age, no association with allergy was found. Food and antibiotic al-

Figure 6. The prevalence of doctor-diagnosed allergy and asthma from questionnaire analysis and serum specific IgE to respiratory allergens in pediatric patients coming to tonsillectomy (Study III) divided by age and the presence or absence of previous adenoidectomy. Children with previous adenoidectomy present with more doctor-diagnosed allergy and asthma but not with more IgE specific for common respiratory allergens than children with no previous adenoidectomy. P-values for comparisons were obtained using the Chi-square test.



lergy was equally common in girls and boys. Bronchial asthma was marginally more common in boys older than seven than in similarily-aged girls 14% vs. 5% (p=0.105) (**Figure 7**).

In control children, no statistical difference was seen in specific IgE to respiratory allergens between females and males. When the data from all children were pooled, males had significantly more often allergen-specific IgE than females (p=0.036 in children >7 years, p=0.035 in children 2-17 years) (**Figure 8).**

Figure 7. History of doctor-diagnosed allergy and asthma in girls and boys who came to tonsillectomy, presented in age groups. Boys older than 7 years have more doctor-diagnosed allergy, allergy to dusts and doctor-diagnosed non-antibiotic allergy. P-values for comparisons were obtained using the Chi-square test.

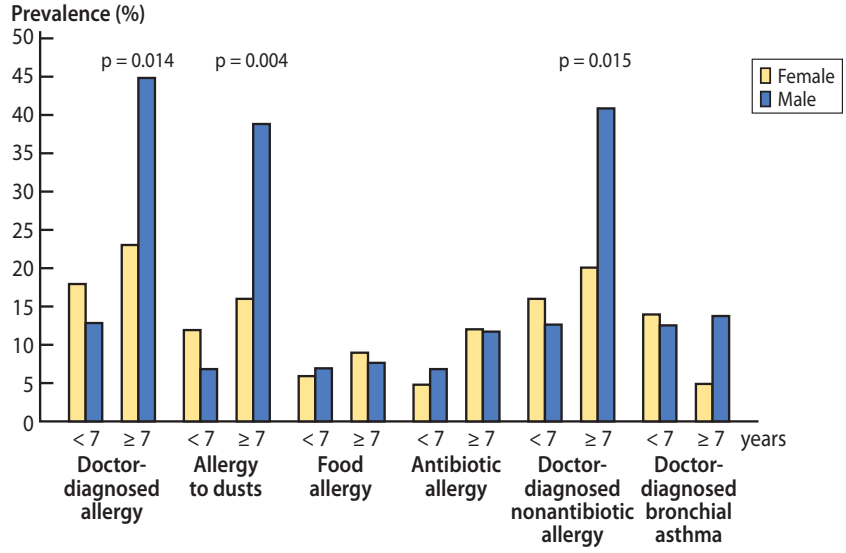
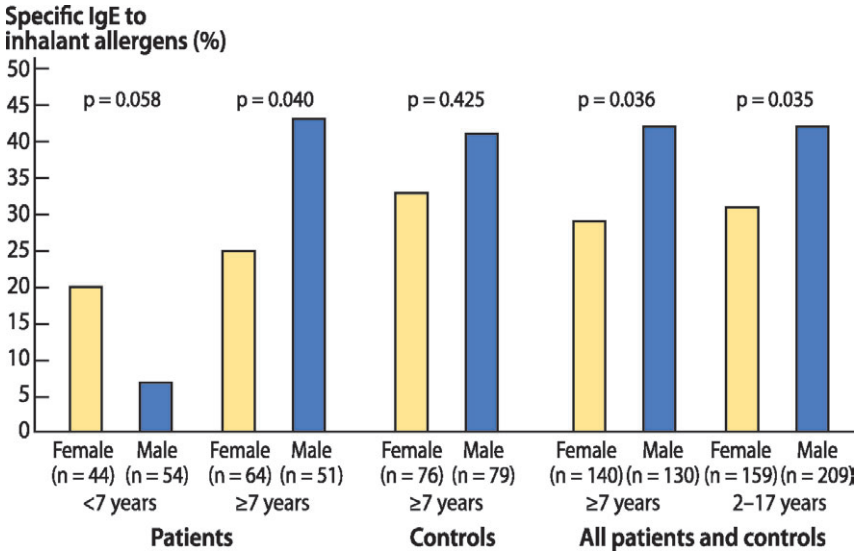


Figure 8. Presence of specific serum IgE against common inhalant allergens in tonsillectomy patients and controls, in different age groups. P-values for comparisons of female and male groups in each age group were obtained using the Chi-square test.



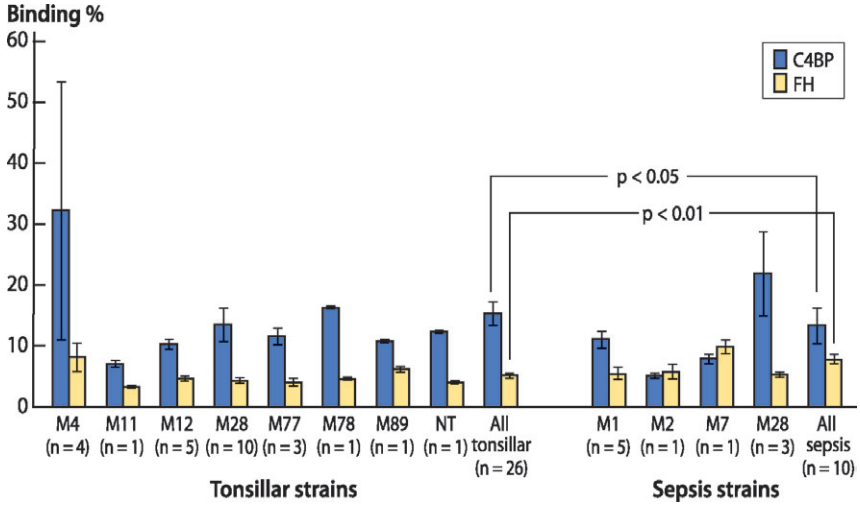
8.2.3 Tonsillar streptococci and binding of complement regulators FH and C4BP

Tonsils are known to harbor potential pathogens even without symptoms of infection. The causes for bacterial persistence and virulence in the tonsils are not well known. Group A streptococci can escape opsonophagocytosis by binding soluble complement inhibitors. Thus it was of interest to find out whether tonsillar streptococcal strains isolated from patients would be able to use host complement regulatory factors FH and C4BP and how this ability would correlate with their pathogenicity and with the studied clinical parameters of the patients.

Bacteriological cultures were performed from the removed tonsils (n=403). Streptococci were isolated from 21% of the tonsillectomy patients. Thus, altogether 79 streptococcal strains were obtained from 403 tonsils removed. Of these, 56 were GAS, four group B streptococci (GBS), seven group C streptococci (GCS) and 12 group G streptococci (GGS). Subsequently, strains from two tonsils of a single patient were treated as one strain. To study the possible immune evasion mechanisms of the isolated bacteria, 26 GAS, two GBS, two GCS and four GGS strains were compared for their ability to bind FH and C4BP. No or only weak FH or C4BP binding ability was seen in GBS, GCS or GGS strains. On the other hand, 6 of the GAS strains were found to bind FH and/or C4BP above the threshold levels. Subsequently, T and *emm* types of the GAS strains were determined. Two GAS of M4 serotype could bind both FH and C4BP. The clinical course of tonsillar disease and operation indication did not correlate with streptococcal strains nor complement regulator binding, or the T or *emm* serotype of GAS.

Since binding of complement regulators has been suggested to be important for bacterial virulence, the ability of tonsillar strains to bind C4BP and FH was compared with 10 GAS strains isolated from bacteremia patients. For this analysis 5 M1 and 5 strains representing other serotypes were selected. Of the bacteremia strains two could bind FH and three C4BP (**Figure 9**). M1 strains were not more efficient in complement regulator binding than the other strains. Tonsillar strains bound more C4BP, and the bacteremia strains more FH. Only marginal statistically nonsignificant correlation of the T or M types of the bacteria with complement regulator binding was observed.

Figure 9. Direct binding of ^{125}I labeled complement regulators FH and C4BP by GAS strains isolated from tonsils and blood (Study IV). Tonsillar strains bound more C4BP, and the bacteremia (sepsis) strains more FH. Only a partial correlation of the T or M types of the bacteria with complement regulator binding was observed. With permission from Elsevier.

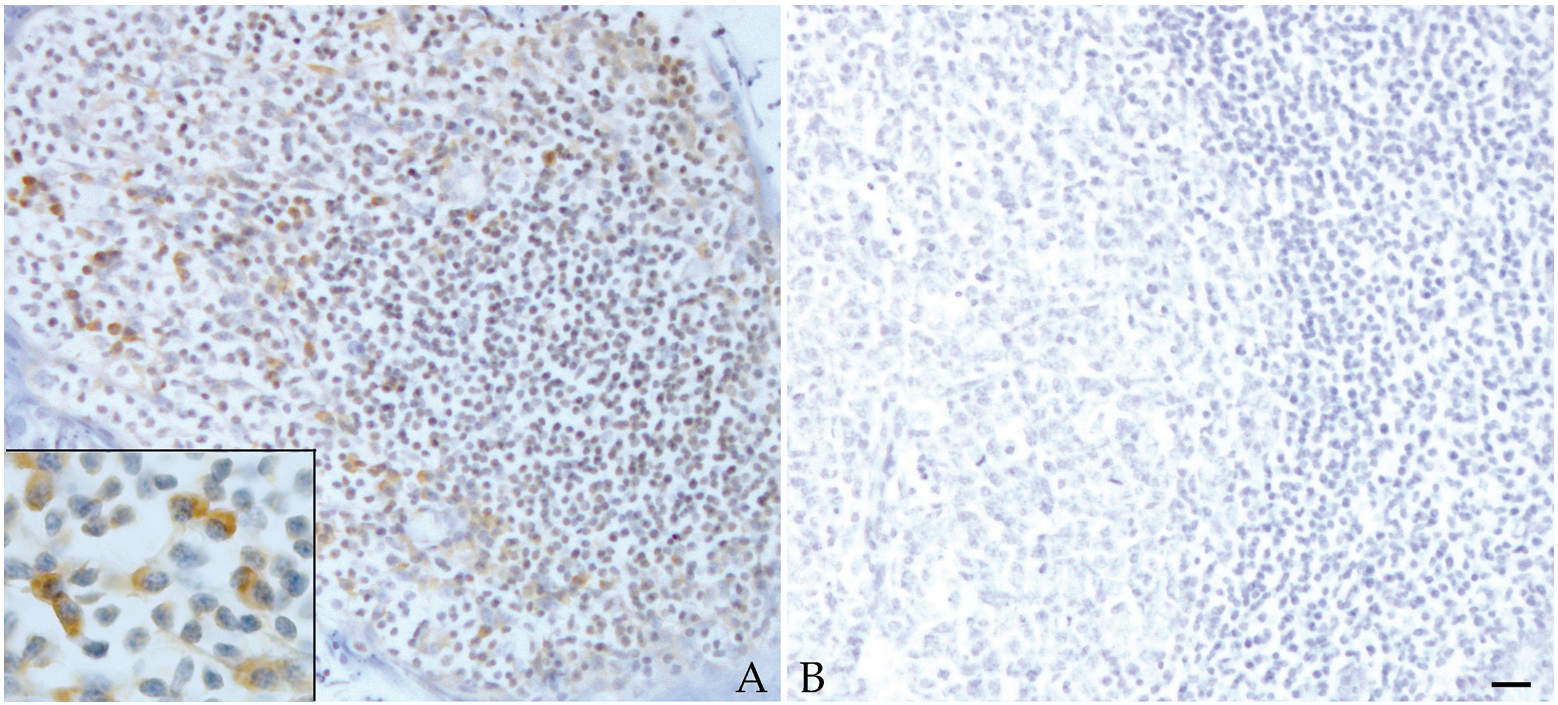


8.2.4 Human rhinovirus/enterovirus (HRV/HEV) in pediatric tonsillar disease

Human rhinovirus is the most common causative agent for respiratory infections and an important cause of asthma exacerbations. It has been earlier found in all other parts of the respiratory tract except the palatine tonsils. To find out if rhinovirus can be detected in tonsillar tissue, we took tonsillar samples from 33 consecutive tonsillectomies at the Clinic of Otolaryngology, Head and Neck Surgery of Helsinki University Hospital, between February and March 2003, outside the primary rhinovirus seasons. Children did not have acute respiratory symptoms at the time of operation.

A positive in situ hybridization result for HRV was observed in 20 (62%) of the 33 specimens. No association was found between a positive HRV result by the in situ hydridization (HRV-ISH) test and patient demographics, history of respiratory diseases, tonsillectomy indication, or positive bacterial or yeast culture from the tonsils. The HRV-ISH signal was localized to epithelial and lymphoid cells in the tonsils (**Figure 10**). For further verification of the findings 5 HRV-ISH negative and 5 positive samples were investigated with a hanging drop-nested PCR method and conventional PCR hybridization assay (PCR-HYB) in a separate laboratory. With the more sensitive nested PCR picornavirus RNA was detected in 9 (90%) of 10 samples. One HRV-ISH negative sample was positive by both PCR methods and only one was negative by all methods. Thus, we could detect HRV-HEV RNA in 75% of the studied tonsils with the methods used.

Figure 10. In situ hybridization for rhinovirus type 14 in a tonsil. Panel A shows detection of rhinovirus-14 with an antisense probe. Positive hybridization signal is visible as cytoplasmic brown reactivity in lymphoid follicle cells. The inset shows a higher magnification to better demonstrate cytoplasmic reactivity. Panel B is a consecutive section from the same specimen probed with a rhinovirus-14 sense probe. No hybridization signal is detected. Bar = 50 µm. (From study V). With permission from Elsevier.



9 DISCUSSION

9.1 Sinonasal diseases

9.1.1 Sexual dimorphism

Females are reported to have more numerous, but less severe infections of the upper respiratory tract and males fewer, but more serious (Falagas et al. 2008). Female patients also report more frequent, numerous and intense other bodily symptoms than males (Bensing et al. 1993). Males after puberty, however, tend to underreport or deny respiratory symptoms, and forget their previous illnesses (Wieringa et al. 1999). As puberty seems to be a crucial time point, some of these differences may be explained by sex-hormones, some by behavioral pressures.

The differences in attitudes towards illnesses may be connected to the development of different forms of sinonasal diseases. Male behavior tends to create delays in seeking treatment, and this may predispose to complications. Female behavior may predispose to repetitive treatments for less severe symptoms. Our results about the history of more frequent mucosal infections in females (**Figure 5**) are in line with previous data, but can be affected by this reporting bias from both sexes (Stalman et al. 2001; Shashy et al. 2004; Falagas et al. 2008).

As an objective proof that different phenotypes in female and male patients do exist, we utilized clinical diagnostic grouping **(Figure 5)** and CT scoring (**Table 8a and 8b**), which both confirmed that males had more mucosal changes in their noses and sinuses. Similar observations have been made in other studies (Chen et al. 2003; Baumann et al. 2007; Busaba et al. 2008a; Busaba et al. 2008b). Both female and male patients with polyposis coming to operation were older than the ones with no polyposis. This suggests that polyposis takes a longer time to develop and that sinonasal polyp disease may just be complicated form of chronic R. This spectrum of disease is also suggested by the latest position paper of R and nasal polyps (Fokkens et al. 2007). However, our results in patients undergoing sinonasal operations suggest that there are considerable differences between female and male genotypes, even when the patients share the same phenotype CRSwNP. In females with CRSwNP, *C4B* deficiencies were very common, but in males very uncommon. The underlying cause for sinonasal polyposis may thus be completely different and independent from the *C4B* deficiencies found.

Clinically, the results suggest that the physicians' approach to female and male sinusitis patients should be different. Mucosal findings in endoscopy and CT scans tend to be more prominent in male patients and associate with sinonasal polyposis. Sinonasal polyposis is a chronic mucosal disorder, and the primary treatment for it is not operative. Operations should be reserved for patients who do not sufficiently benefit from local or systemic steroids, leading to frequent recurrencies of NP. Sadly, operation does not necessarily guarantee better outcome. However, it is possible that in recurrent and chronic R males are not motivated enough to take the prescribed medications and therefore may be in risk for infectious complications. In females who have a lower threshold for physician contacts and who have a lower Lund-Mackay score in CT as a sign of less pronounced mucosal reaction, a decision to operate would call for careful diagnostics and sufficient follow-up with conservative treatment. Both in females and males diagnostic imaging, if conservative treatment fails, is elementary. In females, the goal would be to confirm that the diagnosis is truly a R and not just local swelling unresponsive to antibiotics but causing pressure symptoms. In males, the main indication for imaging would be to rule out complicated and/or polypoid disease.

9.1.2 Complement activation

Interestingly, acute R patients had very high levels of C3, C4 and CH50 as compared with controls. No difference was seen between females and males. This indicates the involvement of complement in inflammation in acute bacterial rhinosinusitis since C3 and C4 behave as acute phase proteins. This further suggests that complement activation could be an important part of the immunological reaction in acute R.

Previously, a higher level of complement factor C4 in male patients with CRS when compared to females has been shown (Ogunleye et al. 2001). This finding was confirmed in this study. The same was found in C3 levels. The higher C4 levels in all male SNO patient groups with no differences in the amounts of *C4* gene deficiencies when compared to controls and female SNO patients suggests an increased complement turnover in male sinonasal disease leading to operation.

9.1.3 Complement *C4* deficiencies

In clinical patient groups, the low number of *C4A* or *C4B* genes was not associated with the circulating C4 levels. Serum C4 protein concentration

reflects only partially the *C4B* copy number as these patients usually have more than 2 *C4A* genes because of gene conversions (Yang et al. 2003). Only if the total number of *C4* genes was 2, we could see an effect on serum C4 level (data not shown). This suggests that more important than total C4 levels in serum is whether the levels of C4A or C4B isotypes are disproportionately low. Due to the poor availability of isotype-specific antibodies that would enable the measurement of C4A and C4B separately, we only measured the levels of total C4 protein.

It is known that C4A binds more avidly to free amino groups on protein antigens and C4B to hydroxyl groups on carbohydrate antigens. The predominant function of C4A is opsonization and immune complex clearance, and of C4B killing of the pathogens by promoting lysis and neutralization (Guerra-Junior et al. 2008). In clinical studies, *C4B* deficiencies have been associated most often with chronic and recurrent viral infections (Pasta et al. 2004; Seppänen et al. 2006). In our study I, *C4A* deficiencies were associated with severe chronic and recurrent R (Seppänen et al. 2006).

C4B deficiencies were associated with chronic and recurrent sinus infections without nasal polyposis (60% CRSsNP patients vs 41% controls, p=0.003) especially in female patients. The highest numbers of total (37%) and partial (74%) *C4B* deficiencies were seen at females with CRSwNP. In females and males, partial *C4B* deficiencies were in the same level in the CRSsNP group (F 60%, M 60%), but possibly due to the small sizes of the male patient and control groups this was significant only in females. Males with CRSwNP had the lowest level of *C4B* deficiencies (0% total, 11% partial) of all patient groups and controls.

It seems that genetic deficiencies of *C4A* and *C4B* genes are associated with different forms of sinonasal disease. *C4A* deficiency is associated with severe chronic sinonasal disease. *C4B* deficiency is associated with CRSsNP in all patients. In female patients with CRSwNP *C4B* deficiency is also associated with a greater risk for disease. In males, the same deficiency may even be protective, or our findings in men were caused by chance due to the small size of the male control group. This suggests that the effect of *C4B* deficiencies to CRSsNP in females and males is similar, but to CRSwNP different. However, no differences between females and males or between patients and controls in NPO group were seen in *C4A* or *C4B* deficiencies suggesting that nasal polyposis without sinus infections is not associated with this deficiency.

9.1.4 Immunoglobulin levels

An IgG subclass level below 2 standard deviations from the mean defines low subclass level. However, due to the lack of published reference materials and universally accepted methods to measure subclasses there is also a lack of universally accepted reference values (Buckley 2002). Clinical consequences of low IgG subclass levels, in the absence of known immunodeficiency, are thus not clear.

Only one patient suffering from a severe primary antibody deficiency was found from our patients coming for sinonasal operation. Different Ig classes and IgG subclasses, defined by their heavy chains, elicit different effector functions. IgG2 and IgG1 are important against purulent extracellular infections, while IgG1 and IgG3 are complement-fixing antibodies that neutralize viruses and further elicit complement-mediated killing of microbes (Avery et al. 2008). Ig isotype-switching is a dynamic process during the immune response, the mechanisms of which in humans are being increasingly understood (Honjo et al. 2004; Avery et al. 2008). Low IgG subclass levels are mainly caused by immune dysregulation associated with a disturbed cytokine balance, leading to subtly impaired Ig isotype-switching. In humans, Th1/Th2/iTreg/Th17 –polarization (involving among others IFNγ, IL-4, IL-17, IL-21, and IL-27) regulates the relative efficacy of isotype-switch to IgG3, IgG1, IgG2, IgA and/or IgE (Avery et al. 2008). Lower, but physiological levels of IgG1 and IgG3 without high levels of IgA and IgG2 in ARS patients may reflect consumption of these Th1-type Igs. The results both in females and males either suggest subtly impaired Th1 responses and IgG1 and IgG3 production, and/or Th2-dominant responses with a relative overproduction of IgA, IgG2 and specific IgE. Normal, even raised, IgG2 and IgA levels in our patients suggest that the great majority of patients admitted to sinus surgery have adequate humoral immunity against common extracellular bacteria (e.g. *S. pneumoniae* and *H. influenzae*) causing purulent infections in the sinuses (Buckley 2002; Bonilla et al. 2005; Avery et al. 2008).

The minor differences in the levels of IgA and IgM between different diagnosis groups of SNO patients are too low to reflect major patophysiological differences between these groups.

9.1.5 Allergic diseases

In SNO patient groups, allergic symptoms were commonly reported (females 58-75%, males 36-75%). In the study by Pallasaho, in Finnish young adults the prevalence of allergic rhinitis or conjunctivitis was 21-28% in females and 21-27% in males; atopic dermatitis 20-25% in females and 13-17% in males; asthma 4-7% in females and 3-10% in males (Pallasaho et al. 2006). Consequently, the prevalence of allergic symptoms and the difference between females and males coming to operation for sinonasal diseases are not different from that of normal population. In ARS patients, allergic symptoms were not commonly reported by females (15%), as compared with males (36%). This may be associated with the age difference (median age 46 years F, 31 years M).

Bronchial asthma was commonly reported by all patients with severe chronic sinusitis (female 55%, male 60%) and in all operated sinusitis patients with polyposis (female CRSwNP 65%, NPO 50%; male CRSwNP 46%, NPO 53%). The reported prevalence of doctor-diagnosed bronchial asthma in the Helsinki area is 7.0% in females and 5.1% in males (Pallasaho et al. 2002). This association of hyperinflammatory nasal disease and hyperreactivity in LRT has also been seen in earlier studies (Fokkens et al. 2007).

Specific IgE to respiratory allergens was found more commonly in male (42%) than female (28%) SNO patients. The female figure of specific IgE is in concordance with a previous report of Finnish females (25%) (Pekkarinen et al. 2007). With another test method, skin prick testing, allergic sensitization was found in 50% of adult males and 44% of adult females (Pallasaho et al. 2006). Our results suggest that respiratory allergy and IgE sensitization are not important susceptibility factors for adult R leading to operation.

9.2. Pediatric tonsillar diseases

9.2.1 Respiratory infections, adenoidectomy and allergy

Adenoid tissue is associated with both the development of allergy and resistance to respiratory infections (Brodsky et al. 1993; Lagging et al. 1998). Viral upper respiratory infections, especially those caused by rhinovirus, induce and exacerbate allergic symptoms and bronchial asthma (Custovic et al. 2005; Heymann et al. 2005; Wark et al. 2005). Conflicting reports about the association of previous adenoidectomy and later development of allergy have been published (Kvaerner et al. 2002; Custovic et al. 2005; Heymann et al. 2005; Wark et al. 2005).

Based on our study, it could be proposed that hypersensitivity disorders and URTI commonly affect the same children. When compared with children with no previous adenoidectomy, doctor-diagnosed allergy and asthma were significantly more common in children who had undergone adenoidectomy and were prone to upper respiratory infections, especially in children under 7 years of age. The most interesting finding of study III was the difference between symptomatic hypersensitivity and bronchial asthma when compared with the presence of specific IgE to common inhalant allergens in the serum, especially in children under 7 years of age. These associations of adenoidectomy, allergy, recurrent infections and asthma in children under 7 years have been recently also confirmed by others (Mattila et al. 2009).

An interesting six-fold increase (from 7% to 43%) of specific serum IgE to common inhalant allergens was seen in boys from under 7 years of age to over 7 years of age. In females of the same age groups the increase was only from 20% to 25%. Similarly, in history of doctor-diagnosed allergy to dusts a 5.5-fold (from 7 to 39%) and in doctor-diagnosed nonantibiotic allergy a 3-fold (from 13% to 41%) increase was seen in boys, but not in girls (from 12 to 16% and 16 to 20% respectively) when children younger than 7 years were compared with school children. In antibiotic allergy, a two-fold increase was seen in both girls and boys, but no differences in food allergy or bronchial asthma were found. These populations are highly selected by their tonsillectomy indications, which is most commonly tonsillar hyperplasia in pre-school children and tonsillar infections in school children. This was not a follow-up study. Therefore conclusions about the significance of this phenomenon cannot be drawn.

9.2.2 Complement evasion by GAS in tonsils

Innate immune responses are raised rapidly against microbes and material from non-viable self-cells, acting even in the most primitive organisms. Thus it would be beneficial for a pathogen to be able to invade host tissue without innate immunity recognition and with less risk for defensive actions from innate and adaptive immunity.

A very effective weapon of target destruction and opsonization is the complement system, which functions both in innate and adaptive immunity. Due to its aggressive self-perpetuating nature it has to be tightly controlled by regulatory membrane-bound and soluble factors to avoid unnecessary damage to host cells. These factors limit C activation in the vicinity of host cells.

Human C response is lured by several pathogens that acquire soluble C regulatory factors from the host. Therefore, in this work the ability of a common tonsillar pathogen, GAS, to bind C regulatory factors FH and C4BP was studied in vitro. Somewhat surprisingly, the binding ability was relatively weak and less commonly found in the bacteria than expected. Furthermore, the occurrence of bacteria with complement regulator binding ability did not associate with any characteristics of tonsillar disease.

When the complement regulator binding strains from tonsillitis patients were tested against strains derived from the blood of septicemia patients, only a very marginal difference in binding of FH and C4BP was seen. When studied if the binding was associated with some specific GAS serotypes, the regulator binding strains were found to be mostly of serotype M1, M4 or M28. The binding varied also within the same serotype. M28 was the most common serotype isolated, as also seen in nationwide survey for the same year (Siljander et al. 2006). In earlier studies, C4BP binding has been seen to some, but not all M4, M6, M8, M11, M18, M28 and M78 serotypes (Perez-Caballero et al. 2000).

These in vitro results suggest that the acquisition of host complement regulatory factors FH and C4BP is not the only virulence factor for GAS that enables it to cause clinical disease in humans. GAS phagocytosis resistance is multifactorial and may not depend on the binding of soluble complement regulators FH and FHL-1 (Cunningham 2000). GAS has also other virulence factors that promote the entry to host cells and increase phagocytosis resistance, which may have a more paramount effect on the tonsillar disease.

9.2.3 Rhinoviruses in tonsils

Rhinovirus is the one of the most common acute respiratory pathogens, and it is responsible for most of the exacerbations of bronchial asthma. In patients with asthma allergen sensitization, high allergen exposure and viral infection were not independent risks for hospital admission for asthma exacerbation, but simultaneously raised the risk for asthma admission ninefold (Custovic et al. 2005). Study number V focused on rhinovirus and showed that during early spring HRV RNA is found also in tonsils. Earlier, HRV RNA has been found in all other parts of the respiratory tract.

February-March is not the peak season for rhinovirus infections (Monto 2002). During the same season, using the same ISH method, HRV detection rates similar to those found in tonsils have been found also in the adenoids (Rihkanen et al. 2004). Therefore, the frequency of the positive detection of HRV/HEV was not a surprise. Other methods used to confirm the primary findings were even more sensitive and revealed more positive results for HRV in tonsils.

The role of HRV/HEV in tonsillar pathology is unclear. It is not known, if tonsillar cells can be infected with HRV/HEV. Thus it cannot be said whether the presence of HRV/HEV in tonsils is just a remnant of previous infection, a sign of an early, yet subclinical new, or even a chronic infection. One reason for the high occurrence of rhinovirus in the adenoid and tonsillar tissues may be related to the immunological function of the human adenotonsillar (AT) tissue. AT is constantly sampling particles passing down the pharyngeal passages for immunological recognition and possibly as a template for antigen production. Therefore, it is natural that many potential respiratory pathogens end up in tonsillar tissue. Why the HRV-infected cells are not destroyed by innate and adaptive immune mechanisms is not known. Alternatively, the viruses could replicate for limited a time until they are destroyed. This would be concordant with the fact that T-lymphocytes from tonsillar tissue have been shown to react to rhinovirus and create a Th1 type response with IFN-gamma and IL-2 production (Wimalasundera et al. 1997).

With the ISH method, HRV-RNA was located to epithelial and lymphoid cells. Rhinovirus RNA is taken into the cells mostly through ICAM-1 receptors. This cell surface glycoprotein is present on APCs, lymphocytes, eosinophils, mast cells, submucosal glands, airway smooth muscle cells, and epithelial cells. It also regulates leukocyte trafficking and accumulation at sites of inflammation. Rhinovirus intake and replication leads to the upregulation of ICAM-1 receptors in epithelial cells (Paganelli et al.

1998). Direct cellular injury caused by rhinovirus infections results in little cellular destruction in experimental infections (Skoner et al. 1995). We could not find any association with detected rhinoviruses and clinical tonsillectomy indications. Rhinovirus infection induces a rapid increase in serum IgE levels without evidence of elevation in the antigen-specific IgE in allergic subjects (Skoner et al. 1995). The role of those high IgE levels is unknown. A synergism between high specific antibody levels with allergic disease and natural virus infection has been suggested (Custovic et al. 2005). Atopic subjects and especially individuals with bronchial hyperreactivity have been recognized to be more susceptible to HRV infections and to more severe cold (Kelly et al. 2008). It is possible that tonsils act as a natural reservoir for HRV between acute exacerbations. Further research is needed to confirm the association of tonsillar rhinoviruses with allergic respiratory disease.

9.3 General discussion: limitations and findings of special interests in the study

In study number I, patients with severe chronic sinusitis were collected retrospectively. There may be a selection bias in the collection of this patient group. However, the inclusion and exclusion criteria applied were the same throughout the study.

In the reporting of medical history, the patients' opinions were trusted and not cross-checked from other sources. Differences between female and male behavior and reporting of their previous illnesses may be confounding factors especially when it comes to previous minor infections in adults. In children, the questionnaires were usually filled by the guardian and it may be possible that accuracy of the history given by the father was not the same as the one given by the mother (Pless et al. 1995).

The control group of males was unfortunately small and, therefore, some of the possible differences may have not reached statistical significance. However, when all available healthy control subjects were pooled, increasing the number of female controls to 134 and male controls to 64, the percentages of *C4* deficiencies and the results remained essentially unchanged.

We did not ask the geographical origin of the patients. It is known that in different parts of Finland, the population selection has led to local enrichment of disease genes (Salmela et al. 2008). However, the genetically most heterogeneous population in Finland is found in the Helsinki capital and the surrounding Uusimaa areas, including people from various ethnic origins.

When studying the associations of clinical sinonasal or tonsillar disease and causative factors one confounding factor may be the difficulty to determine the clinically most relevant indication to operate. Tonsillectomy patients had on average 1.4 diagnoses and also in R one diagnosis does not exclude others. The diagnosis of acute bacterial R in general practice is known to be difficult (Lindbaek et al. 2002). Recurrent R is especially difficult to determine, as it is uncommon to have radiological or other verification for acute episodes.

In R, new EPOS guidelines have stratified the different types of R for clinical use and research **(Table 4)** (Fokkens et al. 2007). The definition for research in the EPOS guidelines accepts that there is a spectrum of diseases in CRS, which includes a polypoid change in the sinuses and/or middle meatus, but excludes those with polypoid disease presenting in the nasal cavity to avoid overlap. Thus CRS with polyposis is considered a subgroup within CRS. Also, recurrent R has been dropped out from the CRS definitions in EPOS. Because of these major changes in guidelines the definitions used in these studies are not fully compliant with the current ones. When the patients for the present study were recruited, the EPOS guidelines did not yet exist. Therefore the American Academy of Otolaryngology-Head and Neck Surgery 1997 criteria were applied as the inclusion criteria (Lanza et al. 1997). However, it is known that the clinical diagnoses are not always as clear-cut as the criteria in guidelines. Overlap between patient groups may exist, and therefore too detailed patient classification must be judged with caution.

We could show that patients fullfilling the criteria for severe chronic R had an increased number of *C4A* deficiencies. Impaired immune complex clearance as a consequence of *C4A* deficiencies after acute sinusitis episode may lead to the development of chronic disease. On the other hand, *C4B* deficiencies, which potentially impair the killing of encapsulated pathogens were associated with CRSsNP. This association was strongest in female patients, who report more recurrent sinonasal infections.

Most interestingly, females and males in the clinically similar group of recurrent or chronic R together with nasal polyposis (CRSwNP) had a very different number of *C4B* deficiencies. This would suggest that the pathogenesis of this disorder might be different in females and males. However, this needs to be confirmed in further studies. The determination of the number of *C4A* and *C4B* genes may help in clinical decision-making. Patients with *C4A* deficiencies should be considered as being at risk for worse outcome and disease progression. Patients, especially females, with *C4B* deficiencies are at risk for recurrent sinus infections, but do not necessarily have the worst prognosis. We did not study direct activation of the different C pathways. As C seems to be upregulated in

sinonasal diseases, and different forms of disease are associated with different C deficiencies, it would be interesting to compare the activation of different C pathways in the different forms of sinonasal diseases.

We did not study the cause for the differences in serum Ig levels of patients. It is known that polymorphisms of several genes regulating TLR signalling and IL levels may result in differences in Ig subclass levels (Kimman et al. 2008).

In tonsillar diseases, the clinical classification is even more difficult than in R, since no commonly accepted guidelines have been presented. Not uncommonly, a child has tonsillar hyperplasia simultaneously with recurrent or chronic tonsillitis, which together lead to the decision to operate. In this study, all indications for the operations were recorded, but the primary indication was systematically determined: hyperplasia if no chronic or recurrent infections were noted, chronic tonsillitis, if not connected with recurrent episodes of acute tonsillitis, and recurrent tonsillitis with recurrent episodes of acute exacerbations. In this way clear and non-overlapping diagnosis groups could be made at least for tonsillar infections. Tonsillar hyperplasia is a common feature also in patients with tonsillar infections. Therefore, these patients were seen also in chronic and recurrent tonsillitis groups. GAS complement inhibitor binding or HRV detection were not associated with different tonsillar diseases or tonsillectomy indications.

It is important to realize that female and male patients not only have different frequency and forms of infectious respiratory diseases, but they also behave differently as a consequence of the disease. Females are more sensitive to symptoms and seek medical treatment more easily. Males tend to underreport, deny and forget symptoms. These differences make it challenging to judge whether conservative treatment has been successful or not and whether operation would be necessary. It is commonly acknowledged that a great deal of operations could be avoided with effective conservative treatments. There is great need for markers to aid clinical decision on operative treatment.

The control subjects in both adult R and pediatric tonsillectomy studies were only collected for serological and gene studies and did not fill out questionnaires. Therefore, we can compare the incidence of symptoms of sinonasal, tonsillar or allergic diseases only between different patient groups and not with control groups. Interestingly, in the incidences of both allergic symptoms and specific IgE sensitization in children coming to tonsillectomy a suggestive sex-linked difference was seen. Although confounding factors are clear (for example, selected populations based on tonsillectomy diagnosis) this suggests that for sex-associated differences

should be taken into account in further pediatric studies on the development of allergy.

Immunity plays a crucial role in infectious and inflammatory respiratory diseases. Usually it is not the infective agent itself, but the immunological defense mechanisms it triggers that cause the patients' symptoms. Different reaction types modulate the disease and are responsible for the variations in prognosis. For the most part, this appears to be the case also in the diseases studied in this thesis.

10 CONCLUSIONS

On the basis of results obtained in this thesis, the following conclusions can be drawn.

1. Sinonasal diseases in adult patients exhibit sexual dimorphism: females more commonly suffer from recurrent infections without polyposis while males develop sinonasal polyposis.

2. Complement system upregulation and possibly activation is an important factor of acute rhinosinusitis in females and males. In males, complement upregulation is also associated with sinonasal diseases leading to operations. The full role of complement in sinonasal diseases remains to be explored.

3. In females, complement *C4B* deficiency and low levels of IgG1 and IgG3 are associated with and may be risk factors for chronic or recurrent adult rhinosinusitis leading to sinonasal operations.

4. In children under 7 years of age, allergies and recurrent infections commonly affect the same individuals. Previously performed adenoidectomy did not have an effect on specific IgE levels to respiratory allergens in children.

5. Binding of complement regulators FH and C4BP in vitro occurs only by occasional GAS strains isolated from tonsillar tissue or blood. Binding did not correlate with the form of tonsillar disease.

6. HRV/HEV is commonly found within tonsils of children. HRV/HEV in tonsils did not associate with any of the studied tonsillar diseases leading to operation.

11 SUMMARY

In the first part of this thesis the association of different forms of sinonasal diseases and plasma concentrations of C3, C4, immunoglobulins, immunoglobulin G subclasses, *C4A* and *C4B* gene numbers were studied in 287 adult patients and 150 sex-matched adult controls. Patients were well characterized and stratified into groups using strict clinical criteria and females and males were also studied as separate groups.

Severe primary antibody antibody deficiencies were rare in patients coming to sinonasal operations. Female patients had more recurrent sinusitis and other mucosal infections and males had more nasal polyposis. Upregulation of complement activity was seen in acute rhinosinusitis patients (high levels of plasma C3, C4, and complement classical pathway activity CH50) and male patients coming to sinonasal operations (high levels of plasma C3 and C4). In females, total and partial *C4B* deficiencies and lower levels of IgG1 and IgG3 were associated with rhinosinusitis leading to sinonasal operations. *C4A* deficiencies were found to predispose to severe chronic rhinosinusitis in females and males. In female patients with chronic or recurrent rhinosinusitis with nasal polyposis *C4B* deficiencies seem to predispose to the disease, but in males with a similar disease *C4B* deficiencies seem to be protective. This suggests a different pathophysiology between sexes in this form of sinonasal disease.

In the second part of this thesis work 213 children coming to elective tonsillectomy were studied and compared with 155 randomly selected school children. An association with recurrent upper respiratory tract infections and hypersensitivity disorders was seen especially in children under 7 years of age. However, this association was not seen in levels of specific IgE to respiratory allergens in the same age group. Both symptomatic respiratory allergy and specific IgE to respiratory allergens became more common in boys than girls over 7 years of age.

We were able to show that although both rhinoviruses and bacterial pathogens were found in the tonsils, no association between their presence and clinical forms of tonsillar disease was seen. The ability of GAS to bind complement regulators FH and C4BP did not differ between strains causing tonsillar diseases or septicemia, suggesting that other virulence mechanisms of the bacteria are more important.

12 TIIVISTELMÄ (FINNISH SUMMARY)

Väitöstutkimuksen ensimmäisessä osassa selvitettiin nenän ja sivuonteloiden tulehdusten ja polyyppitaudin yhteyttä komplementin C3- ja C4-tasoihin, vasta-aineiden ja niiden alaluokkien plasmapitoisuuksiin ja *C4A*- ja *C4B* geenilukumääriin tutkimalla 287 aikuista potilasta ja 150 aikuista kontrollihenkilöä. Potilaat jaettiin ryhmiin tiukkojen kliinisten kriteerien mukaan tutkien naiset ja miehet myös erillisinä ryhminään.

Vakavat vasta-ainepuutokset olivat harvinaisia sivuonteloleikkauksiin tulevilla potilailla. Naisilla oli yleisemmin toistuvia sivuontelontulehduksia ja muita limakalvotulehduksia ja miehillä nenän polyyppitautia. Komplementin toiminnan lisääntymisen merkkejä oli havaittavissa äkillistä poskiontelontulehdusta sairastavilla potilailla (korkeat plasman C3, C4 ja komplementin klassisen tien aktivaation CH50 tasot) ja sivuonteloleikkaukseen tulevilla miehillä (korkeat plasman C3-, C4-tasot). Naisilla täydelliset ja osittaiset *C4B*-geenipuutokset ja matalat plasman IgG1- ja IgG2-tasot liittyivät leikkaukseen johtavaan sivuontelotautiin. *C4A*-geenipuutokset altistivat sekä miehiä että naisia vaikeille sivuontelontulehduksille. Kroonista tai toistuvaa sivuontelontulehdusta ja polyyppitautia samanaikaisesti sairastavilla naispotilailla *C4B*-puutokset vaikuttivat altistavan sairaudelle, mutta samalla tavalla sairastavilla miespotilailla samalla puutoksella oli suojaava vaikutus. Tämä viittaa siihen, että tämä sivuontelotaudin muoto kehittyy eri tavalla naisilla ja miehillä.

Väitöstutkimuksen toisessa osassa tutkittiin 213 nielurisaleikkaukseen tulevaa lapsipotilasta verraten heitä 155 satunnaisesti valittuun koululaislapseen. Toistuvien ylähengitystieinfektioiden ja yliherkkyysoireiden välillä havaittiin yhteys alle 7-vuotiailla lapsilla. Tätä yhteyttä ei kuitenkaan ollut havaittavissa seerumin allergia-vasta-ainetutkimuksessa. Sekä oireinen hengitystieallergia että spesifisten vasta-aineiden määrä seerumissa lisääntyivät kouluiässä pojilla tyttöjä enemmän.

Tutkimuksessa pystyttiin osoittamaan, että vaikka sekä rinoviruksia että tautia aiheuttavia bakteereja on osoitettavissa nielurisoissa, niiden läsnäololla ei ollut vaikutusta nielurisasairauden laatuun. Nielurisoista ja verenmyrkytyspotilailta eristettyjen A-streptokokkien kyvyllä sitoa komplementin säätelytekijöitä FH ja C4BP ei ollut vaikutusta näiden bakteerien aiheuttamien sairauksien kulkuun viitaten siihen, että muilla bakteerin virulenssiin vaikuttavilla tekijöillä on suurempi merkitys.

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Appendix 1

Tutkittavan potilaan kyselykaavake

Tutkittavan koodinumero: _____ Tutkittavan nimikirjaimet: _____

Tutkimus:
POSKIONTELOTULEHDUSTEN JA NENÄPOLYPOOSIN IMMUNOLOGISET TAUSTATEKIJÄT

Sukupuoli □ nainen □ mies Syntymävuosi: 19____ Ikä:____v

Poissulkukriteerit	**Kyllä**	**Ei**
Ikä alle 18v		
Raskaus / tai sen epäily / tai **imettää**		
Kärsii rangaistusta **vankilassa** tai on **asevelvollinen**		
Vajaakykyinen (tutkimuslain vaatima kysymys)		
Kieltäytyy tutkimuksesta		

Sisäänottokriteerit	**Kyllä**	**Ei**
a) diagnoosi		
Pitkittynyt, krooninen poskiontelotulehdus (kesto 3kk ajan tai pidempään)		
Toistuva poskiontelotulehdus (4 kertaa tai useammin vuoden pituisena ajanjaksona)		
Nenän ja/tai sivuonteloiden polyyppitauti		
Poskionteloiden **tietokonekerroskuvauksessa** sivuontelontulehduksen/polypoosin löydökset		
Nenän **tähystyksessä (endoskopiassa)** sivuontelontulehduksen/polypoosin löydökset		
b) toimenpide		
Tulossa **sivuonteloiden endoskooppiseen leikkaukseen (FESS)**		
Tulossa nenän tai sivuonteloiden **polyyppien poistoon**		
Tulossa muuhun sivuontelotoimenpiteeseen:_____		

Poskiontelotulehduksen riskitekijät	**Kyllä**	**Ei**
1. Allergiat (jos vastasitte ei, menkää suoraan kohtaan 3.)		
2. a) Jos allergia todettu, onko diagnosoitu: Missä:_____ Ihotestein: □ Verikokein: □ Oireiden perusteella: □		

b) Jos allergia todettu, mille olette allerginen: _____ _		
3. Onko teitä tutkittu epäillyn allergian takia, mutta allergiaa ei todettu		
4. Tupakoitteko, tai oletteko koskaan tupakoinut (jos vastasitte ei, menkää kohtaan 7)		
5. Jos olette joskus tupakoinut, mutta lopettanut: Montako vuotta on tupakoinnin lopettamisesta: _____v Montako vuotta ehditte tupakoida: _____v		
6. Jos edelleen tupakoitte: Montako vuotta olette tupakoinut: _____v Montako savuketta keskimäärin päivässä:_____kpl		
7. Altistutteko ammatissanne, kotonanne tai harrastuksissanne jatkuvasti **hengitysteitä ärsyttäville aineille** (homesienet, hitsauskaasut, liuottimet jne) Mille:_____		
8. Harrastatteko uintia:_____/ tuntia viikossa tai _____/ tuntia kuukaudessa		
9. Onko teillä todettu **pitkäaikainen nenän limakalvojen ärsytystila** (kutina, aivastelu)? Jos syy tiedossa, mikä:		
10. a) Onko teillä jatkuvaa/toistuvaa **nenän vuotamista eteenpäin**		
b) Onko teillä jatkuvaa/toistuvaa **nenän vuotamista taaksepäin (”takanuhaa”)**		
c) Onko teillä jatkuvaa/toistuvaa **nenän karstaisuutta**		
d) Onko teillä jatkuvaa/ toistuvaa **nenän tukkoisuutta**		
11. Käytättekö **pitkäaikaisesti** (yli 2 viikon ajanjaksoja) **nuhatippoja** Mitä:		
12. a) Oletteko käyttänyt **pitkäaikaisesti** (yli 2 viikon ajanjaksoja) **nenän kortisonivalmisteita** Mitä:		
b) Oletteko käyttänyt **kortisonivalmisteita suun kautta** nenäoireisiinne:___kertaa/vuosi		
13. a) Onko teillä ollut **pitkäaikaista** (yli 3kk) **tai toistuvaa** (yli 4 kertaa/ v.) **poskiontelon tulehdusta.** Minkä ikäisenä oireilu alkoi vuotiaana		
b) Oletteko joutunut syömään **antibioottikuureja nenän/poskiontelon tulehdukseen** Montako viimeisen 12 kuukauden aikana:_____		
c) Onko teitä koskaan aiemmin **leikattu poskiontelotulehdusten** (_____kertaa) **tai polyyppien** (_____kertaa) takia alkaen _____iässä		
Poskiontelotulehduksen riskitekijät	**Kyllä**	**Ei**
14. Onko teillä ollut **korvatulehduksia >3 vuodessa/ liimakorva** (alleviivaa oikea)____iässä		
15. Onko teillä todettu **vino nenän väliseinämä (erottaa sieraimia toisistaan)**		

16. Onko teillä koskaan ollut **nenämurtumia tai poskimurtumia (alleviivaa mitä)**		
17. Onko teillä todettu **nenän alueen kasvaimia**		
18. Onko teillä todettu **ylähampaiden juuritulehduksia**		
19. Onko teillä **astmaa? (jos vastasitte ei, menkää kysymykseen 22)**		
20. Jos astma todettu, onko diagnosoitu: Oireiden perusteella: ☐ Kotona tehtävin puhallustestein: ☐ Sairaalatutkimuksin: ☐ Missä:_____		
21. Jos astma on todettu, onko se kausiluonteinen/ ympärivuotinen/rasitusastma Montako vuotta:_____v Säännöllinen lääkitys ☐ Montako lääkettä:_____kpl Montako lääkettä säännöllisesti: _____kpl		
22. a) Onko teillä ollut **yli viikon kestäneitä nivelsärkyjä tai arkuutta käsissä tai jaloissa**		
b) Onko teille tehty **tutkimuksia niveltulehdusten takia osastolla tai poliklinikalla**		
c) Onko teillä ollut **jänteiden kiinnityskohtien tai jännetuppien tulehduksia**		
23. a) Onko teillä **silmien jatkuvaa kuivuutta tai roskan tunnetta**		
b) Joudutteko **käyttämään silmien kostutustippoja/ vuotavatko silmänne**		
c) Onko teillä **todettu silmien bakteeritulehduksia samanaikaisesti poskiontelotulehdusten kanssa: mitä_____**		
24. a) Onko teillä todettu **vähintään 8x vuodessa (tai 4x/ 6 kk) toistuvaa ☐ huuli tai ☐ sukuelinherpestä**		
b) Onko teillä ollut **runsaasti reikiä hampaissa (kariesta eli hammasmätää)**		
c) Onko teillä **herkästi ientulehdusta (parodontiittia)**		
25. a) Onko teillä ollut **toistuvaa tai kroonista nielurisatulehdusta** (angiinaa)		
b) Onko teille tehty **kitarisaleikkaus**____v. iässä **/ nielurisaleikkaus** ____v. iässä		
26. a) Onko teillä todettu **valoyliherkkyyttä tai aurinkoihottumaa** (alleviivaa kumpaa)		
b) Onko teillä taipumusta **nokkosrokkoon** (urtikariaan)		
c) Saatteko mielestänne **tavallista herkemmin mustelmia**		
27. a) Onko teillä ollut **toistuvia hiivasienitulehduksia** (suussa, sukuelinalueella, jaloissa) Antibioottikuureihin liittyen: ☐ Ilman antibioottikuureja: ☐		
28. Onko teillä ollut **toistuvasti keuhkokuume** (pneumonia)_____ kertaa		

Appendix 2

Tutkittavan potilaan kyselykaavake *Versio 1.0 / 2.1.2000*

Tutkittavan koodinumero: _____ Tutkittavan nimikirjaimet: _____

Tutkimus:
Toistuvien ja kroonisten poskiontelotulehdusten immunologiset taustatekijät

Sukupuoli □ nainen □ mies Syntymävuosi: 19____ Ikä:____v

Poissulkukriteerit	**Kyllä**	**Ei**
Ikä alle 18v		
Raskaus / tai sen epäily / tai **imettää**		
Kärsii rangaistusta **vankilassa** tai on **asevelvollinen**		
Vajaakykyinen (tutkimuslain vaatima kysymys)		
Ollut joskus **toistuva poskiontelotulehdus** (4 kertaa tai useammin vuoden pituisena ajanjaksona)		
Ollut joskus pitkittynyt, krooninen poskiontelotulehdus (kesto 3kk ajan tai pidempään)		
Tämänkertaisen poskiontelotulehduksen oireiden kesto alle 7 vuorokautta		
Tämänkertaisen poskiontelotulehduksen oireiden kesto yli 28 vuorokautta		
Kieltäytyy tutkimuksesta		

Sisäänottokriteerit	**Kyllä**	**Ei**
Positiivinen **poskiontelohuuhtelulöydös**		
Poskionteloiden **röntgentutkimuksessa** nestevaakapinta tai kokonaan varjostunut ontelo		

Poskiontelotulehduksen riskitekijät	**Kyllä**	**Ei**
1. Allergiat (jos vastasitte ei, menkää suoraan kohtaan 4.)		
2. Jos allergia todettu, onko diagnosoitu: Ihotestein: □ Verikokein: □ Oireiden perusteella: □		
3. Jos allergia todettu, mille olette allerginen: _____ _____ —		

Poskiontelotulehduksen riskitekijät (jatkuu)	**Kyllä**	**Ei**

4. Tupakoitteko, tai oletteko koskaan tupakoinut (jos vastasitte ei, menkää kohtaan 7)		
5. Jos olette joskus tupakoinut, mutta lopettanut: Montako vuotta on tupakoinnin lopettamisesta: _____v Montako vuotta ehditte tupakoida: _____v		
6. Jos edelleen tupakoitte: Montako vuotta olette tupakoinut: _____v Montako savuketta keskimäärin päivässä:_____kpl		
7. Altistutteko ammatissanne, kotonanne tai harrastuksissanne jatkuvasti hengitysteitä ärsyttäville aineille Mille:_____		
8. Onko teillä todettu pitkäaikainen nenän limakalvojen sairaus tai ärsytystila Mikä:_____		
9. Onko teillä jatkuvaa nenän vuotamista ja/tai karstaisuutta		
10. Harrastatteko uintia Kuinka monta tuntia :_____/ viikossa tai _____/ kuukaudessa		
11. Käytättekö pitkäaikaisesti (yli 2 viikon ajanjaksoja) nuhatippoja Mitä:_____		
12. Käytättekö pitkäaikaisesti (yli 2 viikon ajanjaksoja) nenän kortisonivalmisteita Mitä:_____		
13. Onko teillä todettu nenäpolyyppeja		
14. Onko teillä todettu vino nenän väliseinämä (erottaa sieraimia toisistaan)		
15. Onko teillä koskaan ollut nenämurtumia		
16. Onko teillä koskaan ollut poskimurtumia		
17. Onko teillä todettu nenän alueen kasvaimia		
18. Onko teillä todettu ylähampaiden juuritulehduksia		
19. Onko teillä astmaa? (**jos vastasitte ei**, tämä oli viimeinen kysymys)		
20. Jos astma todettu, onko diagnosoitu: Kotona tehtävin puhallustestein: ☐ Sairaalatutkimuksin: ☐ Oireiden perusteella: ☐		
21. Jos astma on todettu: Montako vuotta:_____v Säännöllinen lääkitys ☐ Montako lääkettä:_____kpl Montako lääkettä säännöllisesti: _____kpl		

Appendix 3

Tutkittavan potilaan kyselykaavake

Tutkittavan koodinumero: _____ Tutkittavan nimikirjaimet: _____

Sukupuoli □ nainen □ mies Syntymävuosi: 19____ Ikä:____v

Lääkäri täyttää

Poissulkukriteerit	**Kyllä**	**Ei**
Ikä alle 2 tai yli 16v		
Kieltäytyy tutkimuksesta		

Sisäänottokriteerit (vain pääleikkausindikaatio= kyllä)	**Kyllä**	**Ei**
Toistuvat äkilliset nielurisatulehdukset (yli 6/v. tai yli 3/v. kahtena peräkkäisenä vuotena), josta syystä tulossa nielurisaleikkaukseen (tonsillektomiaan)		
Krooninen nielurisatulehdus (krooninen tonsilliitti), josta syystä tulossa nielurisaleikkaukseen (tonsillektomiaan)		
Nielurisojen hyvänlaatuinen suurentuma (tonsillahyperplasia), josta syystä tulossa nielurisaleikkaukseen (tonsillektomiaan)		
Vamman johdosta Lasten ja Nuorten sairaalaan hoitoon tuleva potilas		

Huoltaja täyttää:	**Kyllä**	**Ei**
1.Onko lapsellanne ollut toistuvia ylähengitystietulehduksia?		
2.Onko lapsellanne ollut korvatulehduksia?		
3.Jos lapsellanne on ollut korvatulehduksia, kuinka monta niitä on ollut elämän aikana?		

1-3: □ 4-6: □ 7-10: □ yli 10: □		
4.Onko lapsellanne ollut lääkärin toteamia poskiontelontulehduksia?		
5.Jos lapsellanne on ollut poskiontelontulehduksia, kuinka monta niitä on ollut elämän aikana 1-3: □ 4-6: □ 7-10: □ yli 10: □ jatkuva: □		
6. Onko lapsellanne jatkuvaa nenän vuotamista ja/tai karstaisuutta?		
7. Onko lapsellanne nenäverenvuototaipumusta?		
8. Onko lapsellanne herkästi mustelmataipumusta?		
9. Kuorsaako lapsenne?		
10. Onko lapsellanne selkeitä ja toistuvia unenaikaisia hengityskatkoksia?		
11. Onko lapsellanne lääkärin toteama allergia? (jos vastasitte ei, menkää suoraan kohtaan 14)		
12. Jos allergia todettu, onko diagnosoitu: Ihotestein: □ Verikokein: □ Oireiden perusteella: □		
13. Jos allergia todettu, mille lapsenne on allerginen: _____ Missä allergiatutkimukset on tehty?_____		
14. Tupakoidaanko lapsen kotipiirissä?		
15. Onko lapsenne sairastanut rauhaskuumeen (mononukleoosin)?		
16. Onko muilla perheenjäsenillä toistuvia tai pitkäaikaisia nielurisatulehduksia?		
17. Onko lapsen hoitopaikassa tai ystäväpiirissä toistuvia tai pitkäaikaisia nielurisatulehduksia?		
18. Onko lapsenne päivähoidossa? Perhepäivähoito:□ Tarhahoito□		
19. Onko lapsellanne astma? (jos vastasitte ei, tämä oli viimeinen kysymys)		
20. Jos astma todettu, onko diagnosoitu: Kotona tehtävin puhallustestein: □ Sairaalatutkimuksin: □ Oireiden perusteella: □		
21. Jos astma on todettu: Montako vuotta:_____v Säännöllinen lääkitys □ Montako lääkettä:_____kpl Montako lääkettä säännöllisesti: _____kpl		

KIITÄMME MIELENKIINNOSTA TUTKIMUSTAMME KOHTAAN!