The role of Gastro Esophageal Reflux in Chronic Rhinosinusitis

Elin-Johanne Katle

Thesis for the Degree of Philosophiae Doctor (PhD)
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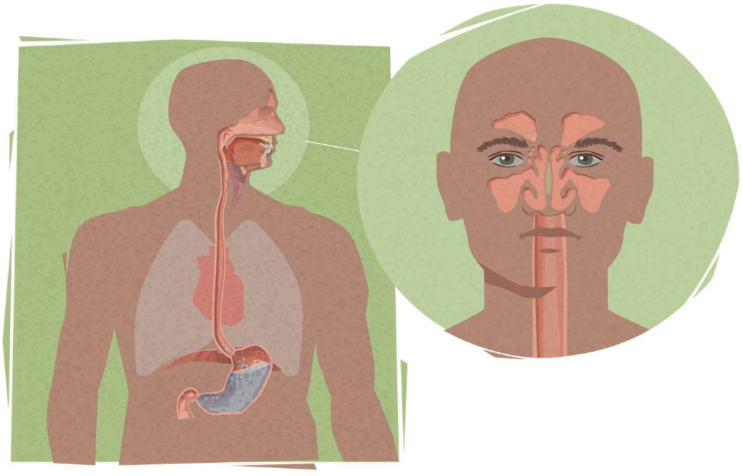

Scientific environment

This work is a result of excellent cooperation between several institutions.

The work of this thesis started in collaboration with my supervisor Professor Sverre Steinsvåg at Department of Otorhinolaryngology, Sørlandet Hospital in Kristiansand and Haukeland University Hospital. The studies have been carried out at Stavanger University Hospital in the Department of Otorhinolaryngology and Section of Gastroenterology in collaboration with my co-supervisor Professor Jan Gunnar Hatlebakk at section of Gastroenterology, Haukeland University Hospital in Bergen.

The Research Department has organized the necessary office facilities at "Forskningens Hus" and "Forskertua". I have participated in the Clinical Immunology Research Group at Stavanger University Hospital. Professor Roald Omdal leads this group, and he has been my co-supervisor. I have worked in the research laboratory at Stavanger University Hospital.

Professor Jan Terje Kvaløy at Department of Mathematics and Physics, University of Stavanger, Norway has been a co-supervisor in the field of statistics.

As a PhD candidate, I have been affiliated to Department of Clinical Medicine, Faculty of Medicine, University of Bergen (UiB).

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Stavanger, March 2019

Elin-Johanne

Contents

1. Abbreviations

ABRS	Acute bacterial rhinosinusitis
AERD	Aspirin exacerbated respiratory disease
AET	Acid exposure time
AR	Allergic rhinitis
ARS	Acute rhinosinusitis
ASA	Acetyl salicylic acid
BMI	Body Mass Index
COX	Cyclooxygenase
CRS	Chronic rhino sinusitis
CRSwNP	Chronic rhino sinusitis with nasal polyps
CRSsNP	Chronic rhino sinusitis sin nasal polyps
CT	Computer tomography
EER	Extraesophageal reflux
EMT	Epithelial to mesenchymal transition
EPOS	European Position Paper on Rhinosinusitis and Nasal Polyps
ERD	Erosive reflux disease
FESS	Functional endoscopic sinus surgery
GER	Gastroesophageal reflux
GERD	Gastroesophageal reflux disease
GerdQ	Gastroesophageal reflux disease Questionnaire
IgE	Immunoglobulin E
H. Pylori	Helicobacter Pylori
IL	Interleukin
LES	Lower esophageal sphincter
LPR	Laryngopharyngeal reflux
NERD	Non erosive reflux disease
NLR	NOD-like receptor
NO	Nitric oxide

NOD	Nucleotide-binding oligomerization domain
NP	Nasal polyps
NSAIDs	nonsteroidal anti-inflammatory drugs
OME	Otitis media with effusions
OSAS	Obstructive sleep apnea syndrome
PCD	Primary ciliary dyskinesia
PND	Post nasal drip syndrome
PPI	Proton pump inhibitor
RDQ	Reflux Disease Questionnaire
SAP	Symptom association probability
SI	Symptom index
SNOT-20	20-item Sino-Nasal Outcome Test
SNOT-22	22-item Sino-Nasal Outcome Test
SCUAD	Severe chronic upper airway disease
Th1	T helper cell type 1
Th2	T helper cell type 2
TLESR	Transient LES Relaxation
UES	Upper esophageal sphincter
VAS	Visual analogue scale

2. Abstract

Background:
Chronic Rhino Sinusitis (CRS) is one of the most prevalent chronic disorders in industrialized countries. The disease has a major negative impact on quality of life, social and professional capacity as well as health in general. The etiology is multifactorial and partly unknown. As patients may have symptoms inadequately controlled, despite treatment as recommended by internationally validated guidelines, there is a need to understand more of this disease. It has been discussed for decades if gastroesophageal reflux (GER) may have a role in the pathogenesis of CRS in some patients.

Objectives:
The main aim of this research was to obtain a better understanding of the role of GER in CRS and to get a final answer to the question: Is there an association between GERD and CRS?

- Investigating the sino- and nasal-quality of life in patients with gastroesophageal reflux disease (GERD) compared to a control population.
- Assessing the occurrence of reflux in CRS-patients with the 24-hour multichannel intraluminal impedance pH-monitoring.
- Evaluating the presence and level of pepsin in saliva and nasal secretions, in CRS-patients compared to age and gender matched healthy controls.

Methods:
Three controlled studies were carried out, one on GERD-patients and two on CRS-patients.

- We used the 20-item Sino-Nasal Outcome Test in GERD-patients and a healthy control group.

- We performed 24-hour esophageal multichannel impedance pH-monitoring in CRS-patients and compared the results with data from a European trial on healthy subjects.
- We used the Peptest®, an assay for detection of pepsin, to evaluate the findings of pepsin in CRS-patients and an age and gender matched group of healthy controls.

Results:

- Patients with GERD had a reduced nose- and sinus-related quality of life compared to a control group based on having a significantly higher total SNOT-20 score. Accordingly, this study indicates that there may be an association between GERD and CRS.
- CRS-patients had significantly higher incidence of gastroesophageal reflux compared with asymptomatic controls as measured with 24-h esophageal multichannel impedance-pH monitoring.
- As measured by the Peptest, we did not find more pepsin in saliva or nasal secretions in CRS-patients than in healthy controls, nor in those with high GerdQ scores or verified proximal reflux. We measured high concentrations of pepsin in both patients and healthy controls. This indicates a limited validity of the Peptest as screening tool for GER in CRS.

Conclusions:

The reduced sino- nasal quality of life in GERD-patients compared to healthy controls indicates a possible link between GERD and CRS.

More reflux in CRS-patients compared to asymptomatic controls and the proximal extent of refluxate further indicates a link.

High concentrations of pepsin in nasal secretion and saliva in both CRS-patients and healthy controls, as measured with Peptest, indicates a limited validity of the test for diagnostic purposes of GERD-induced CRS. The limited validity is further confirmed

by the absent correlation between Peptest and proximal reflux as measured with 24-hour multichannel impedance pH-monitoring.

3. List of publications

I. Katle EJ, Hart H, Kjaergaard T, Kvaloy JT, Steinsvag SK.
Nose- and sinus-related quality of life and GERD.
Eur Arch Otorhinolaryngol. 2012;269 (1):121-5.

II. Katle EJ, Hatlebakk JG, Grimstad T, Kvaloy JT, Steinsvag SK.
Gastro-oesophageal reflux in patients with chronic rhino-sinusitis investigated with multichannel impedance - pH monitoring.
Rhinology. 2017;55 (1):27-33.

III. Katle E-J, Hatlebakk JG, Omdal R, Kvaløy JT, Steinsvåg SK.
Nasal and salivary pepsin as a biomarker for gastro-esophageal reflux in chronic rhinosinusitis.
Rhinology Online. 2019; (2):25-31. DOI: 10.4193/RHINOL/19.003.

4. Background

The role of gastroesophageal reflux (GER) in chronic rhinosinusitis (CRS) has been a controversy for decades. According to the Montréal definition and classification of gastroesophageal reflux disease (GERD) there is a connection between GERD and the airways and a possible connection between GERD and CRS (1). Both GERD and CRS are common diseases, and coexistence by chance is to be expected. In the European Position Paper on Rhinosinusitis and Nasal Polyps from 2012 (EPOS2012), it is concluded that more research is needed to conclude about GERD as a possible causative role in CRS (2).

4.1 The healthy sino-nasal system

4.1.1 Anatomy

The first description of the paranasal sinuses is credited Galen (130-210 AD) nearly two millennia ago with reference to Galen`s "De usu partium" were they are described as the "Porosity of the scull". It was Leonardo Da Vinci (1452-1519) who gave the first detailed description of the anatomy of the paranasal sinuses with illustrations containing descriptions of both frontal and maxillary sinuses (3).

The paranasal sinuses consist of the frontal, maxillary, ethmoid and sphenoid sinuses (4). They all have a communication to the nose (Figure 1).

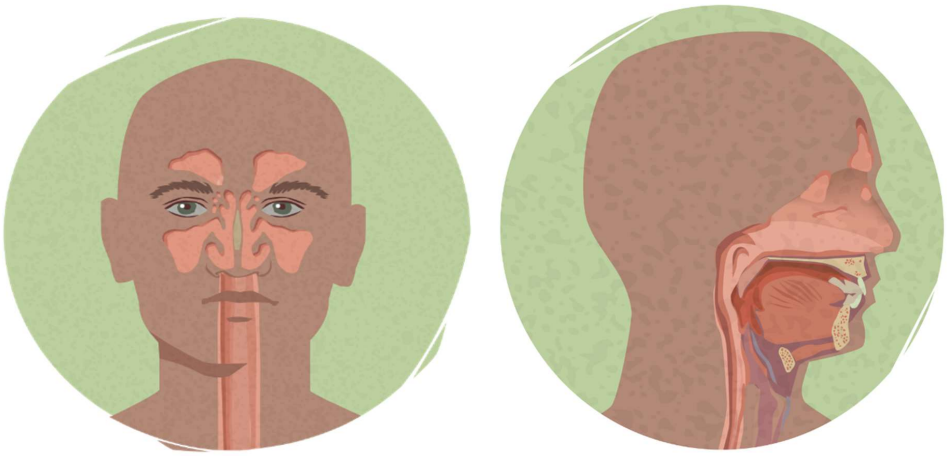

Figure 1. The nose and the paranasal sinuses. The frontal sinuses above the eyes, maxillary sinuses below the eyes, ethmoid between the eyes and sphenoid sinuses behind the eyes in the midline.

The epithelium of the sino-nasal mucosa consists mostly of ciliated columnar cells (80%), but also mucus producing goblet cells and progenitor basal cells are present (5).

4.1.2 The function of the nose

The nose is essential for human health, mainly related to respiration and olfaction. Respiration through the nose gives completely different condition for the lungs compared to the oral alternative. During nasal inspiration, the air is filtered, humidified, tempered and supplied with nitric oxide, enhancing normal lung function.

4.1.3 The mucociliary system

The airway epithelium is covered by a two-layered liquid phase. The periciliary fluid (the sol phase) with low viscosity enabling the cilia to beat without resistance and the mucus layer (the gel face) with high viscosity that traps inhaled particles. This two-layered liquid phase, together with the biphasic ciliary beating, plays an important role in the clearance of the mucosal surface of the nose and sinuses, called the mucociliary transport. This is an effective barrier protecting the airways from

pollutants, allergen and microbes etc., and thereby contributes to prevention of CRS and to proper flow of air (6, 7).

4.1.4 The function of the paranasal sinuses

Why do we have paranasal sinuses? According to Negus in 1957, these air-filled spaces do not have any specific function. They are just evolutionary remnants. (8). However, in "Eighteen hundred years of controversy" regarding the function of the paranasal sinuses (9) tenable theories are identified and discussed.

Resonance to the voice

There is no consistent correlation between the size of the sinuses and resonance of the voice. Some animals with powerful voice have large sinuses, e.g. howling monkeys. On the other hand, lions with small sinuses have powerful voice and guinea pigs while giraffe with relatively quiet voice have large sinus cavities (10).

Humidifying and warming inspired air

The exchange of air in the sinuses during respiration has been suggested to contribute to both warming and humidifying of inspired air. However, as the volume of this are negligible several authors have doubted this theory. In this perspective, the size of the nasal turbinates is of much greater importance (10).

Olfactory function

It has been proposed that the paranasal sinuses are increasing the olfactory area. However, as they are lined by non-olfactory epithelium, this theory is probably false (10).

Thermal insulation

The role of the sinuses in providing thermal insulation to vital parts of the brain, has been suggested, but also dismissed. Eskimos and certain species of monkeys, living in cold climate, frequently lack frontal sinuses while they frequently are large in Africans (10).

Protection against trauma

It has been suggested that the sinuses may have a role in absorbing trauma and thus protect the cerebrum. However, as i.e. moose do not have large sinuses and do sustain high impact trauma without damaging sensory organs, this theory has flawed (10).

Assisting growth

A role of the frontal and maxillary sinuses in the growth and formation of the face, has been debated. However, as people having only a single frontal sinus, do not necessarily show asymmetric facial development for the theory is probably not valid (10).

Reduce the weight of the head

The theory that the air-filled sinuses are lightening the skull to maintain equipoise of the head seems unlikely. Calculations have shown that if the sinuses where filled with spongy bone, the weight of the head would only rise with one per cent (10).

Mucus production

An old theory saying that the physiological purpose of the sinuses is to produce mucus to moisten the nasal cavity is dismissed by the fact that the mucosa in the sinuses contain very few glands and the amount of mucus produced in the sinuses will not be significant (10).

Production of nitric oxide (NO)

Production NO takes place in both the paranasal sinuses and the nasal cavity. Thus, the sinuses are aiding nasal cavity specific immune defense as NO is both virocide and bactericide. NO can upregulate the ciliary beat frequency and thus further support the unspecific mucosal defense both in the upper and lower airways (11). The nasal and paranasal NO production has an important role in ventilation perfusion as the vasodilation effect of NO is enhancing pulmonary oxygen uptake. In patients with primary ciliary dyskinesia (PCD) and in cystic fibrosis, nasal NO is extremely low (12).

Thus, currently, knowledge, it seems that the function of the sinuses simply is to improve the airway function through additive production of NO (Keir 2009).

4.2 Rhinosinusitis

Rhinosinusitis is defined as concurrent inflammation of the mucosal lining the nose and paranasal sinuses. Depending on the frequency and duration, it is defined as acute or chronic (2).

According to EPOS2012, rhinosinusitis is characterized by two or more symptoms, one of which should be nasal blockage/ obstruction/ congestion or nasal discharge (anterior/ posterior nasal drip). There may also be facial pain/ pressure and reduction or loss of smell. These symptoms should be combined with objective signs of disease found by CT scan or endoscopy with/ or without nasal polyps, mucopurulent discharge and/ or mucosal edema of the ostiomeatal complex.

By scoring the answer to the question: "How troublesome are your symptoms of rhinosinusitis?" on a 100 mm VAS scale, the disease can be graded as; mild (0-30), moderate (31-70) or severe (71-100). A VAS >5, indicates a level of disease affecting the patient´s quality of life (2).

4.2.1 Acute rhinosinusitis (ARS)

Definition

Acute rhinosinusitis (ARS) in adults is defined as rhinosinusitis, lasting for less than 12 weeks with complete resolution of symptoms and symptom free intervals if the disease is recurrent.

According to EPOS 2012, ARS also includes common cold, a mild, usually self-limited, upper respiratory illness. Common cold (acute viral rhinosinusitis) is defined to last for less than ten day. Although benign in character, it is a huge economic burden on society due to its commonness, frequent health care visits and absence from work (13). Acute post-viral rhinosinusitis is the condition when there is an

increase of symptoms after five days or the symptoms are persistent after ten days but lasts for less than 12 weeks.

Epidemiology

ARS is a common disease with a prevalence varying from six to 15 % in different studies depending on study parameters (2). The condition is primarily handled in primary care.

Pathophysiology

As ARS is most frequently caused by viruses, and viral infection increase the bacterial adhesion to the mucosa, a very small subgroup of acute post-viral rhinosinusitis has a bacterial supra infection. ARS is normally appearing in this order: viral (common cold), post-viral and bacterial acute rhinosinusitis (ABRS). Only 0.5-2.0 % of patients with viral ARS develop an acute bacterial infection secondarily. In ABRS, there will normally be fever, elevated CRP, severe local pain (unilateral predominance), worsening of sickness after an initial milder period and/ or purulent nasal secretion with unilateral predominance of discolored discharge (2).

The most common viruses isolated in adults with ARS are rhinovirus. Coronavirus is another frequently occurring pathogenic factor. (13). The most common bacteria in ABRS are Staphylococcus pneumonia, Haemophilus influenza, Moraxella catarrhalis and Staphylococcus aureus.

There may be a pathophysiological link between allergic rhinitis (AR) and ARS (14), possibly because of changes in ciliary motility (2). A swelling of the mucosa in the nose and sinuses with reduced exchange of gases between the two compartments may add to this. Cigarette smoking has been suspected to predispose for ARS through the same mechanisms, but there is a lack of evidence for such a connection. However, cigarette smoke has been shown to release inflammatory mediators and alter ciliary beat frequency (15).

Anatomical factors that theoretically could worsen the sino-nasal airflow like septal deviation, Haller cells, concha bullosa, choanal atresia, nasal polyps and hypoplasia of sinuses are more frequent in patients with ARS than in controls (2).

Treatment

Main treatment for most patients with ARS is nasal saline irrigation, nasal decongestants and topical steroids. As ABRS is rear compared to ARS, antibiotics are rarely indicated. In spite of this knowledge, antibiotics are frequently used on these patients both by specialists and by GPs. As increasing bacterial resistance and increasing use of antibiotics are major health problems, it is a paradox that the use of antibiotics in this group of patients is huge, a trend that definitely should be changed. Oral corticosteroids are normally not indicated (2).

A Norwegian group has made a trial on Chloramphenicol eye drops on ARS and found it to be more effective compared to systemic antibiotics (16).

Intranasal corticosteroids may improve ARS and are recommended, twice daily (17). Oral corticosteroids are normally not indicated (2).

Nasal decongestants quickly open a stuffed nose, but it must be used with care due to high risk of adverse mucosal congestion if used for more than seven-ten days (2). Nasal decongestants combined with dexpanthenol has improved the efficacy of symptom relief in ARS (18).

Phenylpropanolamine, a systemic mucosal decongestant, is prescribed by some family doctors, but rarely by specialists. The risk of side effects, such as dry mouth, neurological manifestations and possible a risk of cerebral hemorrhage, must be considered (19).

External or internal nasal dilators may mechanically open the nasal vestibulum and thus improve nasal patency, comfort and sleep quality (20).

4.2.2 Chronic rhinosinusitis (CRS)

Definition

CRS is rhinosinusitis lasting for at least 12 weeks.

Two phenotypes are defined; chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis sine nasal polyps (CRSsNP).

Epidemiology

Chronic Rhino Sinusitis (CRS) is one of the most prevalent chronic disorders in industrialized countries today with major negative impact on quality of life, social and professional capacity as well as health in general (21).

As a global agreement on the definition of CRS is lacking, reliable figures of the prevalence do not exist. However, it is estimated that CRS affects 15.5 % of the total population of United States (22). This makes CRS the second most prevalent among chronic diseases (2). The prevalence of CRS in urban population in Europa is between 5-15% (21) and approximately 12 % of Americans below 45 years have CRS (23, 24). Lange et al found the prevalence of CRS to be 9 % all over and up to 4 % affected by sino-nasal polyps (25).

The diagnosis of CRS cannot be determined by symptoms alone. It requires an investigation by specialist with CT scan or nasal endoscopy. Hence, it is likely that the diagnosis is underscored. Some epidemiological studies are based on symptom questionnaires only, and thus might be of limited reliability, difficult to assess and can give an overestimation (26). However, using the EPOS definition for questionnaires in epidemiological studies has a high reproducibility and a good correlation with endoscopic findings (27).

Severe Chronic Upper Airway Disease (SCUAD)

SCUAD is the term defining those patients with chronic upper airway inflammation and symptoms inadequately controlled despite treatment according to internationally validated guidelines. The term includes conditions like non-allergic rhinitis, allergic

rhinitis, occupational rhinitis, aspirin exacerbated diseases and recalcitrant CRS. Comorbidity are common in these patients and might increase the severity.

SCUAD seems to be an underestimated medical problem, and there are gaps in knowledge of the disease and underlying mechanisms. However, it is stated that patients suffering from SCUAD should be handled with special attention because of risk factors, severity and possible novel treatment alternatives (Bousquet, Bachert et al. 2009) (Prokopakis, Vlastos et al. 2014).

It is a hypothesis that these patients with severe disease in spite of guideline-driven treatment could share a common underlying pathophysiology. More research is needed in understanding these patients and optimize the treatment (28).

CRS-patients as a group has impaired quality of life (29), and patients with SCUAD are assumed to suffer most.

Pathophysiology

CRS can be divided in subtypes, with pathological, etiological and clinical findings in common. The two phenotypes CRSwNP and CRSsNP are recent examples of such. Classifications based on underlying pathophysiological mechanisms, endotype classification, may open up for better understanding of multifactorial characteristics of the disease (30).

Several contributing factors to CRS have been proposed:

Bacteria

Bacteria have been considered being a part of the pathogenesis in CRS in some patients, but their role is still rather unclear. ARS as initiator of CRS is a speculation. Bacteria in biofilm are believed to play a role in recurrent exacerbations (2).

Biofilms

Biofilm is an aggregation of micro-organisms, often bacteria, on a surface, covered by a slimy matrix (31). The extracellular matrix, produced by the micro-organisms, consists of polysaccharides, lipids, proteins and DNA and is contributing to

adherence between bacteria and to the surface of biological or non-biological material (32). This structure is protecting the bacteria from the surroundings, making them more resistant to i.e. antibiotics and the immune system. The biofilm is useful or harmful depending on where it is and what bacteria it contains. In human diseases, especially chronic infections, biofilm can complicate treatment, and as much as 80 % of chronic infections may be caused by biofilms (33).

The establishment and cycle of biofilm may be described in five steps:

- Bacteria settle reversibly on a surface
- The binding to the surface becomes irreversible through production of polysaccharides
- Extracellular polymeric substances surround the bacteria
- The colony of bacteria is maturing as it increases in size and canals for communication are established in the matrix
- As the biofilm reaches a “critical mass” where parts will have insufficient nutrition, parts of the biofilm will detach and might start a new biofilm (33).

The presence of bacterial biofilm is reported in sino-nasal mucosa. As it is present in a high proportion of CRS-patients (32) it is suspected to be involved in pathogenesis of CRS in some patients. The biofilm in CRS is adding more inflammation and can damage the mucociliary system (6). The containing microorganisms are more resistant to antibiotics, and biofilm indicate a poorer prognosis. Staphylococcus aureus seem to be the dominant bacteria in biofilms in CRS, and the one associated with inadequate effects of medical and surgical treatment. More rarely Pseudomonas aeruginosa biofilm is present, and further reduces the potential for successful treatment (34).

There may be an underlying mucosal deficit predisposing Staphylococcus Aureus to colonize intranasal polyps (35). Biofilm adherent to a disrupted epithelium is

associated with high number of immune cells and a potential role in the pathogenesis of CRS (36).

Viruses

It has been hypothesized that viruses may be involved in the pathogenesis of CRS by being a source of chronic inflammation, but the evidence is scant, but viruses may trigger the first inflammatory process that pre-dispose for CRS and contribute to acute exacerbations of the disease (2, 37).

Fungi

Once, there was a theory that excessive immunological response to airborne fungi was the main pathological mechanism of CRS, the "Fungal Hypothesis of CRS". This theory has later been more or less abandoned, as it is not supported by scientific investigations (2). But still, there are associations between fungi and CRS, probably due to local immunological or allergic reactions (38).

Mucociliary dysfunction

Primary ciliary dysfunction

Primary ciliary dysfunction (PCD), including Kartagener`s disease, is a rare condition caused by more or less impaired ciliary motility due to more or less lacking dynein arms. The disease is an autosomal recessive genetic disorder and the diagnosis is based on electron microscopic investigations of mucosal specimens. CRS is common when there is a ciliary malfunction with negative impact on mucus transportation (6).

Secondary ciliary dysfunction

Secondary ciliary defect is caused by a variety of pathogens including viruses and bacteria (6).

Cystic fibrosis (CF) may be regarded as a secondary ciliary dysfunction. Defect chloride channels in the cellular membranes and impaired salt balance in the respiratory secretions makes the latter viscous and difficult to transport. This creates a secondary ciliary problem. CF patients are prone to severe CRS (7).

Allergy

Nasal challenge with allergens may induce inflammation in the nose and the sinuses in patients with allergic rhinitis (AR). Besides, some patients with severe perennial AR may fulfil the diagnostic criteria for CRS, and the prevalence of both conditions is correspondingly increasing. However, AR has not been established as a predisposing factor for CRS (28).

Aspirin (ASA)-exacerbated respiratory disease (AERD)

There is a group of patients with CRS, many of them even suffering from asthma, that experience exacerbation of their diseases when they are exposed to aspirin. Many of them are inadequately controlled and categorized as SCUAD (26). Eosinophilic mucosal inflammation and nasal polyps characterize CRS with AERD. Aspirin and other NSAIDs exacerbate the condition, but the disease persists without exposure to NSAIDs. AERD occurs in 5-10 % of CRS-patients However, in CRSwNP it occurs in 15%-40%. The role of aspirin as a precipitating factor is still unclear as reliable diagnostic tests for aspirin hypersensitivity are not available. Desensitization to aspirin is offered at some clinics, but the effect is disputed, and there is no consensus about treatment protocols. Due to high incidence of cross-sensitivity, all non-steroidal anti-inflammatory drugs that inhibit the cyclooxygenase (COX) enzyme might cause this kind of inflammation due to increase in leukotriene production and decrease in prostaglandin production (39).

Anatomical factors

There are anatomical variations that may contribute to the development of CRS due to narrowing of spaces in nose and sinuses. These are nasal septum deviation, concha bullosa and displaced processus uncinatus. However, studies do not conclude about the pathogenic role of these variations in CRS (2).

Genetic factors

Data on genetic associations are sparse except for CF and primary ciliary dyskinesia (24).

Pregnancy

Pregnancy rhinitis with nasal congestion is common during pregnancy, affecting as many as one in five pregnant women (40). Less is known about the association to CRS (2).

Environmental

Exposure to environmental toxins/ irritants like tobacco smoke and air pollutants, diesel exhaust in particular, promote epithelial damage and airway inflammation. A causal relationship between cigarette smoke and lower airways has long been established, but relationship to disorders in the upper airways has not been completely understood. However, the incidence of CRS is increased among smokers, probably due to negative impact on the mucociliary system (15). Smokers also respond less favorably to surgical measures against CRS (41).

The correlation between air pollutants and CRS is best seen in CRSsNP (42).

Occupational

As there normally is long latency between occupational exposure and symptoms from the airways, a correlation might be difficult to understand and detect. When the inflammation and disease is established, it is often chronic. Though reducing occupational exposure, the symptoms often persist. Absence from work combined with reduced symptoms may indicate an occupational relationship such as in baker's rhinitis (43). Baker's rhinitis is well documented, but data on baker's CRS is sparse.

Immunodeficiency

CRS is more common in patients with an immunodeficiency compared to others. This is specifically seen in IgA deficiency disorders and is correlated to low of count of CD4 positive cells as in AIDS (2).

Immunological

Understanding the immunological mechanisms behind CRS is improving. T helper cells are regulating immunological responses resulting in predominantly neutrophilic or eosinophilic inflammation. Natural T helper cell type 1 (TH1) with neutrophilia

are slightly more frequent in CRSsNP than in CRSwNP and named non-type 2 immune response (30). Natural T helper cell type 2 (TH2) with eosinophilia are dominant in CRSwNP (44) and these type-2 immunological reactions in the mucosa have led to classification of the disease into groups according to the underlying type-2 immune response: Cytokine-based inflammations, eosinophil-based inflammation, IgE-based inflammation and cysteinyl leukotriene-based inflammation. Differentiation based on type of cytokines may currently be most useful in endotyping as there may be opportunities for cytokine specific treatment (30).

Interleukin 5 (IL-5), a type 2 cytokine, is a key mediator in eosinophil activation that has long been associated with allergic diseases and has been a target in treatment of allergic asthma. Now it seems that IL-5 also is a key factor in the pathogenesis of CRS and there are ongoing studies on treatment of CRS targeting IL-5 (45).

Staphylococcus Aureus plays a role in the development of CRS possibly by inducing superantigens and colonization in middle meatus. There is an increased prevalence of intranasal colonization of S. Aureus in CRSwNP, up to 87% compared to up to 33% in healthy controls (46).

Staphylococcal enterotoxins (SE) are stimulating release of cytokines, such as IL-5. SE induced inflammation is present in large subgroups of CRSwNP (47).

Three endotypes of inflammation are defined: Non-type 2, moderate type-2 and severe type-2 inflammation. Non-type 2 is typically found in CRSsNP, having a low risk of asthma and a low risk of recurrence, opposite to severe type-2 being involved in CRSwNP including high risk of asthma and a high risk of disease recurrence. Serum biomarkers, distinguishing between non-type 2 and type 2 CRSwNP are identified (30) with potential therapeutically implications in the future.

A Swedish group has investigated the presence of NOD-like receptors (NLR) that are capable of trigging an immune response. They found higher density of NLR in nasal polyps compared to normal nasal mucosa. When treating with nasal corticosteroids,

the level was normalized. Thus, they suggested that NLR might have a role in CRSwNP (48).

Epithelial-to-mesenchymal transition (EMT)

The molecular mechanism behind polypogenesis has not been fully understood, but resent studies suggest that a hypoxia-induced EMT may be an underlying mechanism. EMT is a biologic process in epithelium enabling the epithelium to assume a mesenchymal cell phenotype with degradation of underlying basement membrane, giving it new properties with an enhanced migratory capacity and invasiveness. When this disruption of normal barrier function occurs, there may be a stimulation of further inflammation with mucosal hyperplasia and subsequent polypogenesis.

Surgical intervention prevents the recurrence of nasal polyposis by allowing re-oxygenation. Hence, this supports that hypoxia has a critical role in the disorder.

EMT correlates to severe CRS (49-51).

GERD

See chapter 4.5.8.

Diagnostic methods in CRS

According to EPOS2012, the diagnosis of CRS should be based on anamnesis, clinical examination and imaging.

Symptoms/ Questionnaires

The patients should be evaluated for nasal congestion, anterior and/ or posterior nasal drip, facial pain/ pressure and reduction/ loss of smell and taste.

Among questionnaires available for symptom evaluation are; SNOT-20 (52), SNOT-22 (53) and the VAS-CRS (2).

Endoscopy

Anterior rhinoscopy and endoscopy for investigation of the nasal cavity and is mandatory for the diagnosis. It includes investigation of the anatomy of the internal

nose, the mucosal characteristics, and assessment of the middle meatus and ostiomeatal complex regarding discharge and polyps (4).

CT-scan

The Lund Mackay score is a widely used scoring system for CT-staging of CRS. The paranasal sinuses (frontal sinus, anterior ethmoidal cells, posterior ethmoidal cells, maxillary sinus, sphenoid sinuses) is scored, each side separately, according to; no abnormality, partial opacification or complete opacification (0,1 or 2 points). The ostiomeatal complex is scored according to; open, partly obstructed or obstructed (0.1 or 2 points). The total score will then be between 0 and (Table 1) (54).

Table 1. The Lund-Mackay scoring system for CT of the sinuses

Sinus system	Right	Left	
Maxillary	0-2	0-2	
Anterior ethmoidal	0-2	0-2	
Posterior ethmoidal	0-2	0-2	
Sphenoidal	0-2	0-2	
Frontal	0-2	0-2	
Ostiomeatal complex	0-2	0-2	
Total Score	0-12	0-12	0-24

Each sinus group is scored between 0 and 2 (0: represents no abnormality, 1: partial opacification and 2: total opacification) and ostiomeatal complex scored between 0 and 2 (0: open, 1 partly obstructed and 2 obstructed).

The Lund-Mackay Staging System was developed to facilitate decisions about treatment of CRS. Originally, it included symptom scores, endoscopic scores and radiologic staging, but it is the radiologic staging that has become widely used (55).

Treatment

There are different therapeutic approaches to CRS due to different phenotypes and endotypes of the disease. CRSsNP and CRSwNP may respond differently to

treatment and endotyping may in the future give more opportunities for more individually targeted treatment.

The treatment of choice can be pharmacological or surgical or a combination of the two, depending on the etiology and severity of the disease. A combined medical and surgical approach is frequently used, but despite maximum effort, there are many patients without cure or symptom relief.

The main objective of medical treatment is to reduce inflammation.

The main objective of surgical treatment is to open narrow spaces, improve sino-nasal communication and to remove pathological tissue.

Medical treatment

Glucocorticosteroids

Glucocorticoids, administrated locally or systemically, reduce airway eosinophil infiltration through activation of intracellular glucocorticoid receptors.

The efficacy of steroids is highest in the endotypes with inflammation type 2 (mainly cytokine based), i.e. CRSwNP. However, CRS-patients of other endotypes may also benefit from steroids.

Intranasal corticosteroids are the preferred initial treatment for both CRSwNP and CRSsNP. In the former, drops may have superior effect on nasal volume and sense of smell compared to the spray due to higher steroid concentration and accessibility to the middle meatus and thus reduce the need for surgery (56). The application technique of the spray appears to be important for its effectiveness. The recommended technique is: blow the nose, shake the corticosteroid nasal spray bottle, bend the head forward, hold the spray bottle in the opposite hand to the nostril, place the tip of the spray bottle just inside the nostril and spray while breathing normally. Do not sniff while spraying as this may cause the medication to pass straight through the nasal cavity and may be swallowed (57).

Studies on systemic steroids in CRSsNP are limited, but in CRSwNP, there are beneficial effects. The risk of systemic side effects limits the indication to severe cases for short-term use (30).

Saline Irrigation/ Douching

Nasal irrigation with isotonic or hypertonic saline solution is recommended in CRS. Xylitol irrigation has, in some studies, given better improvement of symptoms as compared to saline irrigation alone (58).

Long-term antibiotics

Long-term treatment of CRS-patients with macrolide, seems to act primarily through immune-modulation rather than anti-microbial mechanisms. There are diverging results of studies, but patients with normal serum IgE and CRSsNP are more likely have an effect of 12 weeks on macrolides than atopics and those with polyps. The option should be reserved for those refractive to other alternatives (59). In CRSwNP, the documentation of beneficial effect is lower, but Doxycycline has shown promising result in some studies (60). The risk of bacterial resistance must definitely be kept in mind when considering this alternative.

Proton Pump Inhibitor (PPI)

According to EPOS2012, there is not documentation supporting a general recommendation for PPI in treating CRS in adults (2). Further, it has been stated that PPI is not effective in treating extraesophageal reflux if the reflux is nonacidic or nonacidic components are causing the problem (61). There appears to be a subgroup of asthmatics who benefit from PPI without having classical symptoms of GERD (62). Studies on treating laryngopharyngeal reflux (LPR) with PPI have shown improvement of nasal symptom (63). Generally, with PPI for extraesophageal reflux it takes high doses for a long period of time to have an effect. A double-blind trial on patients with both LPR and CRS with 20 mg Omeprazole daily for eight weeks, reduced signs and symptoms of both diseases (64).

Aspirin desensitization

Aspirin desensitization is not recommended as a routine treatment due to lack of evidence and risk of adverse reactions (39).

Biologicals

A trial with anti-IgE on CRSwNP, and comorbid asthma, reduced nasal symptoms, reduced nasal polyps, reduced Lund-Mackay scores and reduced the need for surgical and traditional medical treatment (65).

Recently, Mepolizumab, an interleukin inhibitor previously used in severe eosinophilic asthma reduced size of nasal polyps, reduced nasal symptoms and reduced need of surgery in patients with severe CRSwNP (45).

Current data after phase one and two studies of biologics on CRSwNP corresponds to maximum effect of short-term oral glucocorticosteroids treatment. Unlike the effect of corticosteroids, the effect maintained for a long period. Symptom relief and increasing quality of life came along with polyp reduction. As type 2 targeting biologics may be particularly efficient in type 2 CRSwNP, these patients should be identified (30).

Surgery

Though there is a lack of high-level evidence, functional endoscopic sinus surgery (FESS) is regarded as safe and effective in treatment of CRS when medical treatment has failed and it is justified by the patient's symptoms (2). Surgery is more effective on nasal congestion and facial pain than on loss of smell and nasal secretion.

Long-term outcome of surgery is better in CRSsNP compared to CRSwNP, were the polyps frequently reoccur. The surgery can have different approaches depending on the disease, from mucosa sparing surgery in mild cases of CRSsNP to extended surgery with removal of all visible pathology in more severe CRSwNP (30).

4.3 The Upper Gastrointestinal System in Health

4.3.1 The Esophagus

The esophagus is a tubular organ consisting of longitudinal and circular muscle extending from the upper esophageal sphincter (UES) to the lower sphincter (LES). The sphincters are muscular rings giving narrow parts proximally and distally. Additionally, there is a narrow section at the level of the aortic arch (66) (Figure 2).

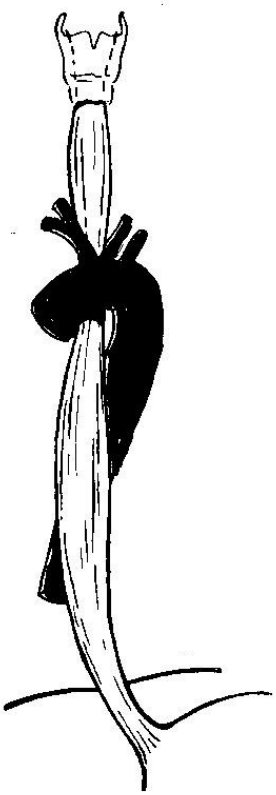

Figure 2. The esophagus with three narrow parts; lower esophageal sphincter (LES), upper esophageal sphincter (UES) and at level of the aortic arch.

Striated muscle fibers dominate the upper third of the tube, while there is smooth muscle in the distal two thirds. Stratified squamous epithelium, innervated by nerve fibers going superficially, covers the lumen and makes the esophagus more sensible to acid compared to the stomach having more protection against acid. There are a few

mucus-secreting glands in distal part of the esophagus. The esophagus has a rich blood supply.

Due to peristalsis in the esophagus and relaxation of sphincters, there is active transport of food and drink to the stomach, while retro-peristalsis will for allow vomiting.

Ructus, defined as containing only air, is a physiological process to prevent the body from being filled up with air, but there can be a hard balance between the physiological reflux of air and pathological liquid reflux. As transport mechanisms will allow ructus to evacuate air, mainly after meals, an imbalance in this mechanism may cause mucosal damage (66).

4.3.2 Stomach

The stomach consists of two parts, the corpus/ fundus and the antrum. The production of acid from parietal cells and pepsinogen from chief cells take place in the corpus/ fundus part where the food is stored and mixed with secretions.

In the antrum, the food is kneaded into smaller particles before entering the duodenum.

Enterochromaffin-like cells, stimulated by gastrin, synthesize and secrete histamine, which stimulates production of acid and pepsin prior to food entering the stomach. The process may be elicited purely by the sight or taste of food. A vagus-mediated relaxation provides an extension of the stomach volume, and thus prevents the pressure in the stomach from rising higher than the pressure in LES when food enters.

Bile and pancreatic enzymes may enter the stomach retrograde.

4.3.3 Pepsin

The name of the proteolytic enzyme pepsin originates from the Greek word for digestion. It is responsible for the first breakdown of proteins in the food to peptides in the stomach and is considered to be the most aggressive proteolytic enzyme in

gastric juice (67). It originates from pepsinogen, secreted from the chief cells in the stomach, and is converted to pepsin in the acidic environment in the stomach. Pepsin is highly sensitive to acid and most harmful in its acidic state at pH 2-3. However, it can be active and cause injury up to pH 6.5. Though not being active when pH is between six and eight, it is not de-naturalized, and can be reactivated. This implies that when pH in refluxate is above 4, and thus not detected by pH monitoring, there can still be active pepsin in the refluxate. Besides, theoretically, inactive pepsin can be reactivated and become harmful by a decrease in pH due to acid drinking or later acidic reflux events (68).

A mucous-producing lining is protecting the stomach from auto-digestion from pepsin. In contrast, neither the squamous tissue lining the esophagus, nor the surface of the airways do have this protective layer. This gives sensitivity to refluxate, and a risk of injury of epithelia of both esophagus and epithelium of the airways. The nasal mucosa may have even less protective capacity against the refluxate and thus, is even more sensitive to its injurious influence.

In extraesophageal tissue, pepsin may be endocytosed into epithelial cells and has been shown to give internal cell derangements (69).

Pepsin is a group of pepsin molecules with small differences in molecular characteristics (Figure 3).

Figure 3. The enzyme pepsin.

Pepsin is actually a group of pepsin molecules, isoenzymes with small differences in molecular characteristics. As the human pepsin A, is produced in the stomach only, and there is no production of pepsin A elsewhere in the body, findings of this in the esophagus, mouth or respiratory tract, is a diagnostic biomarker of gastroesophageal gastric reflux. Different isoenzymes of pepsin A can be found in the gastric juice, and normally pepsin 3a, 3b and 3c, comprises up to 80% of the total enzymes secreted (70). The problem with using pepsin assays in the diagnostic workup of reflux has been lack of specificity to pepsin A of the available assays. Other isoforms of pepsin are also produced in lungs, pancreas (71).

4.3.4 Antireflux mechanisms

These are the mechanisms protecting against reflux of gastric content, and which may explain why it is not even more frequent, and harmful.

Saliva and mucus production

A few mucus-producing glands in the distal few centimeters are responsible for a very limited mucus production in the esophagus. This tiny mucus production in combination with swallowed saliva may neutralize acid reflux. Furthermore, there are mechanisms inducing increased saliva production in response to reflux, giving even more neutralization. The symptom of hyper-salivation is called “water brash” (72, 73). As tobacco smoking decreases the salivary bicarbonate production, smoking will reduce this protective capacity (74).

Peristalsis and swallowing

The movements in the esophagus induced by swallowing is primary peristalsis. Swallowing will work against reflux, starting at the top, and thereby move refluxate distally in the esophagus and drain it back into the stomach. So-called secondary peristalsis can start more distally than during swallowing and drains food from the esophagus and refluxate towards the stomach. Such secondary peristaltic movements are reflex mechanisms initiated by large amounts of refluxate expanding the esophagus. Hence, these mechanisms are working e.g. if having reflux when lying flat sleeping (66).

Lower sphincter function

The muscular tone in LES is an important anti-reflux mechanism. When the resting pressure in the LES is higher than in the stomach, it will protect against reflux. Normally, the smooth muscle of LES will be a part of the gastroesophageal junction, as the smooth muscle of LES will be an intrinsic factor and the surrounding diaphragm, will work as an external sphincter (66).

Upper sphincter function

The upper sphincter (UES) is also a part of the antireflux system, but the knowledge of its function is more diffuse. It appears to be regulated together with the LES, allowing ructus to be evacuated. When the UES is closed, as it normally is, it prevents reflux to the airways (66).

Crus dexter of the diaphragm

The only muscle of the diaphragm that is in direct contact with the wall of the esophagus, is named the crus dexter diaphragm (crural diaphragm) (Figure 5, chap 4.4, page 44). Unlike the smooth muscle of LES, it is striated. Forming a loop at level of the LES, its contraction will protect against reflux when there is a higher pressure in stomach compared to LES. Respiration, cough or heavy lifting that increases the abdominal pressure are typical situations where this mechanism comes into action. Usually, there will be a simultaneous contraction of the diaphragm (75).

His angle

His angle is the name of the sharp angle between the stomach and the esophagus. When there is a distension in the stomach due to a meal, the distal esophagus will be squeezed by the stomach and support the LES in protecting the esophagus from reflux when the stomach pressure is elevated.

4.4 Gastroesophageal reflux disease (GERD)

Normally, there is a balance between injurious forces, the acidic refluxate with varying potency of damage, and defensive forces which include the sphincters, esophageal acid clearance and mucosal integrity, resulting in no injury or symptoms due to reflux of stomach contents (76). Gastroesophageal reflux (GER) may be a physiological and normal event occurring occasionally in all people, without symptoms or complications. In patients with GERD, this balance is disturbed. Several factors may be involved and, although poorly documented, a number of behavioral aspects such as smoking, heavy meals before bedtime, obesity and specific foods may affect this balance (76).

The most common typical presentation of GERD is heartburn and regurgitation (77).

Definition

GERD is defined as a condition that develops when reflux of stomach contents causes troublesome symptoms and/ or complication. The purpose of this phrasing is that the diagnosis should include both patients with symptoms only and patients without symptoms but with complications, irrespective the technology used to achieve it. (1). Troublesome symptoms are normally defined as symptoms of reflux at least once weekly (78).

The manifestations of disease are divided into esophageal and extra-esophageal syndromes (1) (Figure 4).

Esophageal Syndrome

The Montral definition and classification of GERD defines the esophageal syndromes, previously called the classical manifestations, as both symptomatic syndromes with typical symptoms of regurgitation and heartburn and syndromes with esophageal injury. The typical syndrome with esophageal injury is reflux esophagitis, but may also include reflux stricture, Barrett`s esophagus and adenocarcinoma (Figure 4).

The former is further divided into erosive reflux disease (ERD) or non-erosive esophageal reflux disease (NERD) depending on whether there is visible damage to the esophageal mucosa or not.

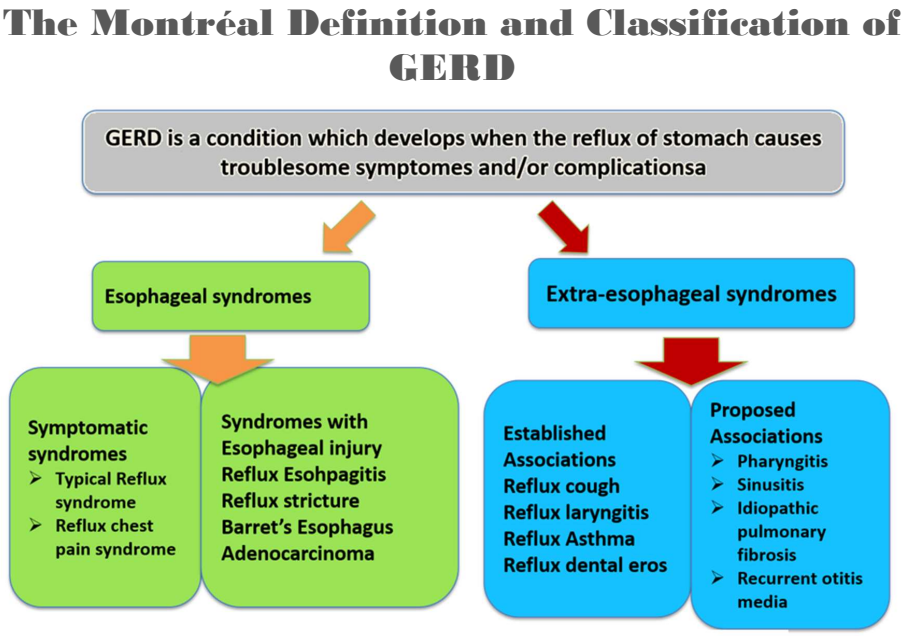

Figure 4. The subdivision of GERD according to The Montréal Definition and Classification of GERD (1).

Extraesophageal Reflux (EER)

Small episodes of EER may damage mucosa of the airways, but there can be numbers of acidic GER episodes in persons without the typical symptoms of GERD (79, 80). Gastric reflux entering the laryngopharynx has also been termed laryngopharyngeal reflux (LPR).

Unlike the esophagus, extraesophageal tissue do not have the surface protection against refluxate. Further, the peristalsis of the esophagus will rapidly return refluxate, particularly after meals, to the stomach. Thus, the esophageal peristalsis will shorten the time of harmful exposure to the surface. The extraesophageal tissue

does not have this protection, and an episode of reflux is likely to influence the surface for a longer period of time (69). It has also been suggested that EER is mediated by pepsin and that typical GERD is mediated by acid. This difference in pathophysiology might explain why PPPs fail to rapidly improve EER disease (69).

Extraesophageal syndrome can occur concomitantly with esophageal syndrome or alone. As much as half of patients with EER, do not present with the typical symptoms of GERD (81), but extraesophageal symptoms alone. This may give a delay in both diagnosis and appropriate treatment.

According to the Montréal definition and classification of GERD, the extra esophageal syndrome may be divided in two groups; the extraesophageal syndromes with an established connection to GERD and the extra-esophageal syndromes with only a proposed association. GERD has an established association to cough, laryngitis, asthma and dental erosions and a proposed association to pharyngitis, CRS, idiopathic pulmonary fibrosis and recurrent otitis media (Figure 4) (1). See chapter 4.5 for further details.

Epidemiology

GERD is a common disease in western countries with a prevalence that may be as high as 20-40 % involving a spectrum of symptoms of some severity possibly related to reflux, typical symptoms of GERD and symptoms of EER (82). The prevalence may be increasing over time (83). The Norwegian HUNT study found increased prevalence of GERD between 1995-1997 and 2006-2009, symptomatic weekly GERD increased by 47% (84).

When defined as at least weekly symptoms of heartburn and/ or acid regurgitation, the prevalence of GERD is reported to be 10-20 % in western countries, and in Asia less (85).

There is a tendency of increasing prevalence with age, but the effect of both age and gender is unclear (86, 87). There is a tendency that a genetic component predisposes. The relationship with obesity is clear and might be the explanation of more GERD in

America compared to Europa (78). Furthermore, the Norwegian HUNT study shows more GERD among smokers (86).

Mechanisms of reflux

Hiatus hernia

When having a hiatus hernia, the LES and crus dexter diaphragm is separated (Figure 5). Thus, a contraction of the crural diaphragm will squeeze the bottom of the hiatus hernia, and not support the LES. After a meal, there will normally not be food in the hiatus hernia. However, when the intraabdominal pressure increases due to e.g. cough or heavy lifting, acid from the acid pocket in the hiatus hernia will easily reflux to the esophagus. In this situation, a contraction of diaphragm and crus dexter, will not give a protection. Due to these mechanisms, the hiatus hernia will predispose for reflux and GERD (88).

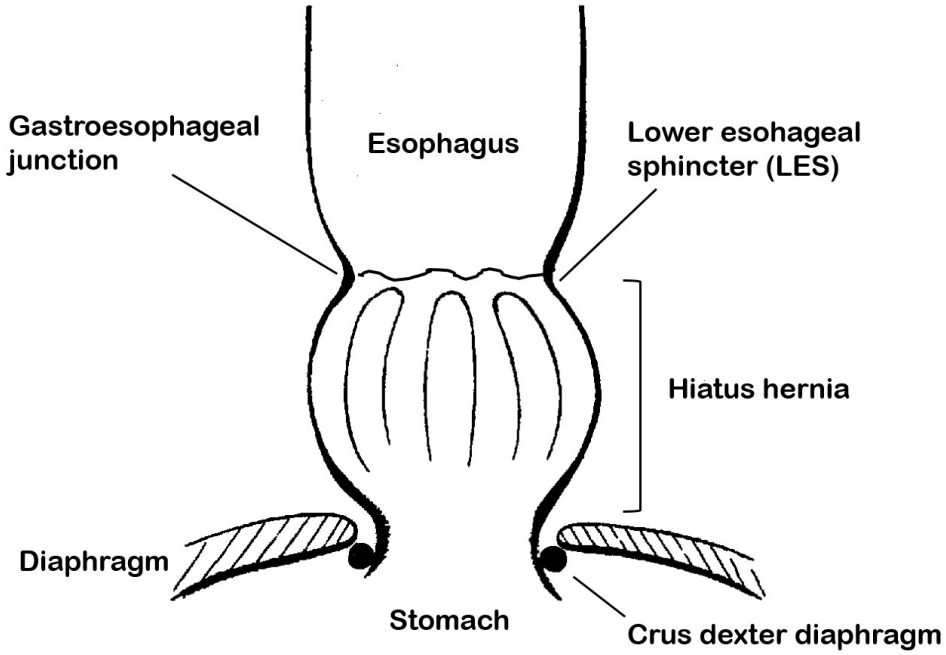

Figure 5. Hiatus hernia. The gastroesophageal junction and LES do not have the same location as the diaphragm. Hence, they are not collaborating against reflux.

Transient LES Relaxation (TLESR)

A transient relaxation of LES is a normal physiological event, mainly to allow ventilation of air and happens independently of swallowing of food. A CNS mediated vaso-vagal reflex stimulated by dilatation of the ventricle may be the mechanism. This happens typically postprandially but can occur whenever during a day. The TLESR lasts for 20-30 seconds and is most important in low-grade GERD.

Hiatus hernia is associated with an increased incidence of TLESR (Figure 5).

Abdominal Pressure

When abdominal pressure exceeds the pressure in LES, the result can be reflux. Increased abdominal pressure will for example happen when coughing or making heavy lifting. Visceral obesity and external pressure on the stomach may further increase the risk.

Low LES Pressure

Reduced pressure in LES gives a risk of reflux as the abdominal pressure may exceed that in lower LES and thus allow ventricular content to pass. This also includes nightly reflux.

A number of triggers have the potential of reducing the tension in LES. Food, medication and hormones are examples of such. Tobacco smoking is also lowering the sphincter pressure (87).

Acid pocket

Particularly after a meal, there will be an accumulation of acid in upper part of the stomach covering the food. This is called the acid pocket, often having a pH between 1 and 2. The acid pocket is close to the esophagus, and as it is in a liquid state, it easily refluxes in to the esophagus resulting in very acidic refluxate.

Impaired nervus vagus activity

The vagal nerve is important for normal motoric activity in esophagus and ventricle (66) and there are speculations that an impaired nerve function can be a cause of reflux due to increased sympaticus activity.

Diagnostic methods in GERD

Symptoms

Heartburn and regurgitation are the most typical symptom of GERD and as troublesome symptoms meets the criteria for the diagnosis of GERD, questions about these symptoms could be diagnostic, and be a legitimate basis for a trial of medical therapy. However, studies have shown that physicians underestimate the importance of symptoms reported by patients, and they may overlap with functional dyspepsia (77). Hence, symptoms of reflux are best assessed with validated patient-reported outcome instruments.

Questionnaires

Structured questionnaires may reduce variability between physicians and between patients in interviews, and many questionnaires about GERD are available. Most of them were originally developed and validated for the monitoring of symptoms in treatment trials (77).

Reflux Disease Questionnaire (RDQ) (89) and Gastro Esophageal Reflux Disease Questionnaire (GerdQ) (90) (91) are validated questionnaires used in diagnostic workup of GERD and in evaluating effect of treatment. GerdQ is derived from RDQ and is easier to use (92). A randomized clinical trial compared a GerdQ-based algorithm with an endoscopy-based approach for the diagnosis of GERD. They found that by using the GerdQ, the health care costs were reduced without loss in diagnostic efficacy. Using GerdQ for the diagnosis of GERD, with a cut-off level of eight in total score, gave a sensitivity of 65% and a specificity of 71% (90).

All the symptom-based questionnaires rely on medical history with the presence or absence of typical symptoms and may not discover atypical cases with extra esophageal syndromes. As a large proportion of patients with EER, lack the typical symptoms of GERD with heartburn and dyspepsia (93), the questionnaires may not be useful in these cases as the sensitivity may be low.
Effective questionnaires for the diagnostics of EER have not been developed.

Thus, the patient´s self-reports about GERD must be treated with caution and recommended to be supplemented with objective measures, dependent of the severity of the disease.

PPI test

PPI test, treatment with PPI followed by evaluation of effect, has been used for diagnostic purposes for GERD. One approach has been to give PPI for four weeks and evaluate both the effect of initiating PPI and the effect of withdrawal. The effect on classical GERD symptoms appeared already after three days. The sensitivity in this study was good, more than 80% responders, but the specificity was low. Some responders did not fulfil the criteria for the diagnosis of GERD, and the response might be due to effect on functional dyspepsia (94). It is also found that symptom response to a two-week course of 40 mg esomeprazole does not add diagnostic precision (95).

Generally, PPI tests are considered as unreliable for the diagnosis of GERD (77).

Gastroscopy

Upper Gastro-intestinal endoscopy has high specificity in diagnosing GERD (96). However, the sensitivity is low, as it will only include patients with visible esophageal lesions, missing the cases of non-erosive reflux disease (NERD). The findings on gastroscopy are classified according to the Los Angeles Classification of the esophagus (LA). This classification is grading the mucosal pathology in four groups from A to D. A is defined as one or more mucosal breaks less than five mm long and not extending between the tops of the mucosal folds, and D is defined as mucosal break involving at least 75% esophageal circumference (97).
The presence of visible manifestation of esophageal pathology in classical GERD may be as low as 50% (77, 98) and even suspected to be lower, especially in EER.

Manometry

Manometry is standardly performed prior to pH and impedance pH-monitoring to plan the placement of the probe. The manometry will further evaluate the motility and peristaltic function of the esophagus and the tonus of LES and UES.

Esophageal pH-monitoring

Routine clinical pH measurement is performed with a pH sensor only in the distal esophagus, and it does not allow characterization of the proximal extent of the refluxate. 24-hour pH-recordings of the esophagus were for many years the gold standard in diagnostic workup of GERD. However, the technique does not allow registration of non-acid reflux, and the rate of false negative, but also false positive tests may be as high as 30% (99). Proximal esophageal and pharyngeal pH monitoring has been performed. However, the reproducibility is low, and it is not used routinely (100).

The total number of reflux episodes and cumulative time when the esophagus is exposed to acidic refluxate are important indicators of the competence of the antireflux barrier (101). There is also a lack in utility of pH monitoring as non-acidic reflux and gas-reflux may not be detected by this technology.

Esophageal impedance pH-monitoring

Impedance monitoring of the esophagus is the latest diagnostic method for evaluating gastroesophageal reflux. Combined with pH measuring, it currently regarded as the diagnostic procedure of choice for the entity by many clinicians and investigators. It is the most sensitive method for detection and characterization of GER. Twenty-four-hour multichannel intraluminal impedance pH-monitoring can detect both acid and non-acid reflux, as well as the number of events and their proximal extent (102, 103).

Intraluminal impedance monitoring allows detection of gastroesophageal reflux based on changes in resistance to electrical current flow between two electrodes, when a liquid and/ or gas bolus moves between them (Sifrim, Castell et al. 2004).

This system includes a portable data-logger with impedance-pH amplifiers, and a single use catheter. The catheter contains one or two pH-electrodes, often one at the tip (gastric) and always one positioned five cm above the LES. There are usually eight electrodes, typically positioned 2, 4, 6, 8, 10, 14, 16 and 18 cm above the LES giving the opportunity of measuring impedance at six different levels in the esophagus (Figure 6).

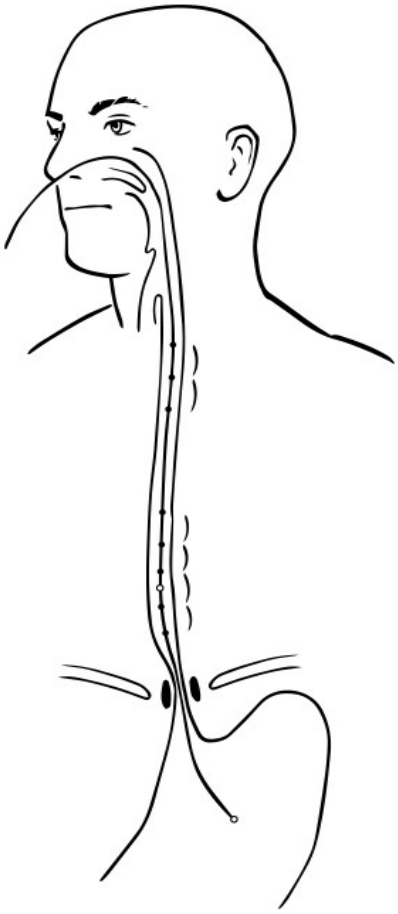

Figure 6. The impedance catheter with eight electrodes is positioned 2, 4, 6, 8, 10, 14, 16 and 18 cm above LES, giving the opportunity of measuring impedance at six levels in the esophagus.

This allows for the evaluation of the distal and the proximal extent of reflux episodes. The amplifier delivers an AC voltage with a frequency between 1 and 2 kHz and measures variations in impedance measures in response to changes in luminal diameter. As air has low electrical conductivity, it will provoke rapid and pronounced rise in impedance in contrast to liquid, which will lower impedance. Dilation of a cavity with fluids will therefore reduce impedance. The change in impedance due to movement of liquid and air in the esophagus is the principle behind impedance monitoring (Figure 7a-7d). Segmentally, the resistance to an electric current due to detection of reflux of air and liquid.

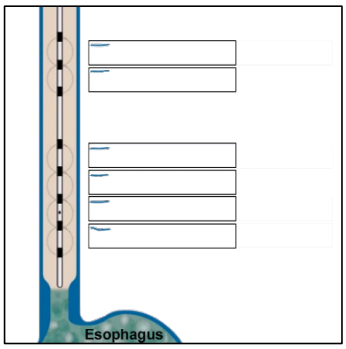

Figure 7a. Impedance monitoring of the empty esophagus. No liquid in esophagus gives low conductivity between electrodes and high impedance at all levels.

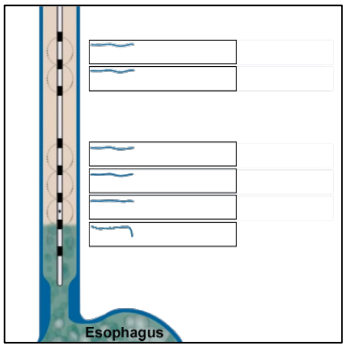

Figure 7b. Impedance monitoring of distal reflux. Reflux in the distal esophagus gives high electrical conductivity between the electrodes and the impedance falls in the distal segment.

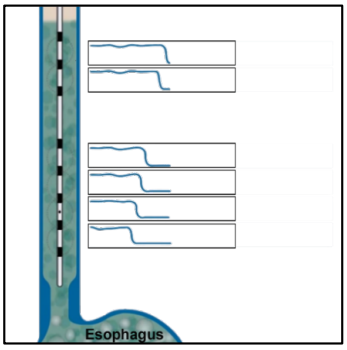

Figure 7c. Impedance monitoring of esophagus when the refluxate has reached the proximal esophagus. Drop in impedance in the mid and upper esophagus indicates proximal liquid.

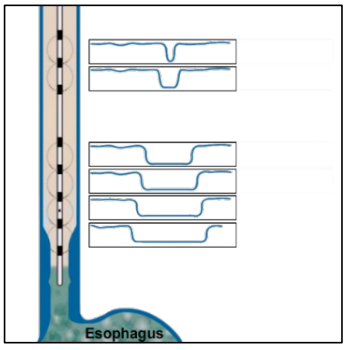

Figure 7d. The impedance measuring in esophagus falls as liquid returns to the stomach.

Measuring pharyngeal reflux has been attempted in studies, but the investigational techniques are difficult in the presence of air and poorly reproducible. While 24-hour impedance pH-monitoring studies of esophagus have shown good reproducibility, both proximally and distally, the monitoring of impedance in upper airways is not reproducible (103, 104).

The number of episodes detected in healthy subjects by impedance at all levels are relatively similar in different studies in Europe and USA (102-104).

It has been debated to what extent automatic analysis is reliable and comparable to manual editing. A software program must be able to distinguish between impedance changes due to real reflux and changes due to non-reflux phenomena such as swallowing or artifacts of in intra-esophageal impedance. Software has improved over the years and currently performs well this (ref. by Albert Kramer at VCM Medical, The Netherlands, personal communication).

A reflux episode may be defined as proximal when the event first is detected as distal reflux, the decline in impedance is >50% and must progress to at least one of the two proximal impedance segments. It is still necessary to confirm such a finding by visual control by a trained clinician or technician. In clinical practice, this may not be routine, but for research, this is mandatory, due to somewhat imprecise detection by commercially available software.

Impedancemetry is used to calculate several parameters, including total number of reflux events, and the subgroups according to posture and pH. This is likely to be the best documented and reproducible variable that can be extracted from a 24-hour recording. Bolus exposure, defined as cumulative duration of decline in impedance to <50% of resting impedance, may be a less reproducible parameter.

Detection of pepsin

As diagnostic methods for GER, are either invasive, expensive or have low sensitivity and specificity, there has been a need for alternative approaches. Detection of pepsin is a novel diagnostic method.

Pepsin has been found in lungs, saliva, nose, sinuses, middle ear and exhaled breath condensate and this has been assumed to represent reflux of pepsin from the stomach. The utility though, of detection of pepsin as a diagnostic marker, is not established (67, 105). As pepsin actually is a family of isoenzymes, and the fact that different isoenzymes seem to have different characteristics and may even be produced at different location, it has been recommended to distinguish between the isoenzymes when testing. As available pepsin assays might not be specific for pepsin A, the isoenzyme found only in the stomach, there might be a limited validity of pepsin as a biomarker for gastric juice and hence for GERD when used on saliva (71).

Different methods for pepsin detection exist.

Peptest® (RD Biomed Ltd, Cottingham, UK) is a commercially available in-vitro diagnostic test for detection of pepsin in saliva. It is an enzyme immunoassay based on a lateral flow device technology. As Peptest is specific to pepsin A, the isoform that exclusively has been found in the stomach, there has been great expectations for the usefulness of the test. The sensitivity and specificity were said to be 88 and 87 % respectively (106). It has been claimed that the test lacks sensitivity for predicting pathologic reflux in children (107) and a recent meta-analysis suggested the sensitivity and specificity to be lower, 64% and 65% respectively (108).

According to the manufacturer, pepsin analysis is to be performed on 1 ml saliva samples delivered by subjects immediately after symptoms, immediately after waking up in the morning and/ or one hour after meals, twice during the day. The glasses contain 0.5 ml µl of 0.01 M citric acid for conservation and the glasses are recommended to be stored in a refrigerator until analysis are performed within seven days (106). A control band on the device indicates correctly performed test and a second line indicates presence of pepsin in the sample. The lower limit for detection of pepsin with Peptest is 16 ng/ ml. The intensity of the detection line is proportional to the quantity of pepsin as read by the reader, LFDR101 (109). A study using another assay for pepsin measuring concluded that pepsin concentration varied during

the day and emphasized the importance of measuring pepsin in relation to the reflux symptoms (110).

Mucosal impedance (MI)

Esophageal mucosal impedance (MI) is a novel diagnostic method based on knowledge of dilated intracellular spaces (DIS) due to acidic-peptic injury to the apical surface of the mucosal epithelium because of reflux. It measures the chronicity of reflux by assessing changes in esophageal MI pattern independent of mucosal erosions and is highly specific for reflux. MI may be a tool for assessment of reflux in suspected EER in future (111).

Treatment

Treatment guidelines recommend life style interventions, over-the counter drugs, antacids, H_2 -receptor antagonists, PPIs and more seldom surgery (Figure 8) (112).

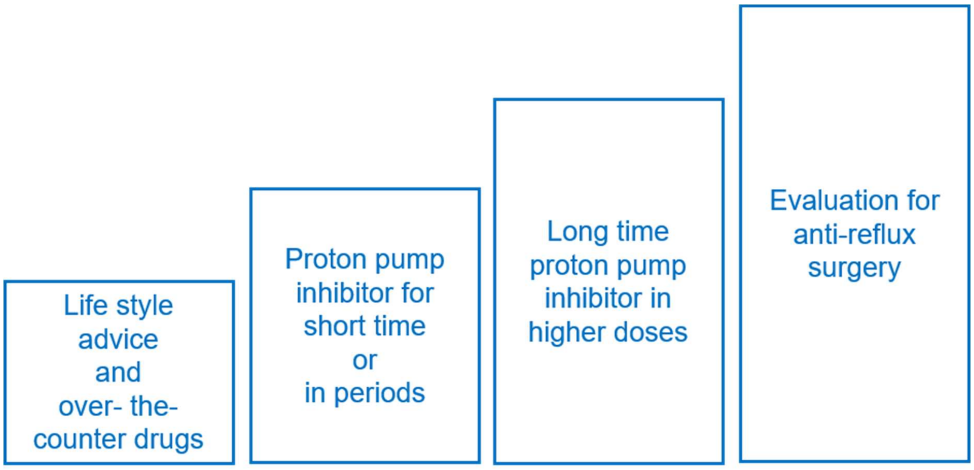

Figure 8. Treatment of diagnosed GERD. H_2 blockers and Alginates can be used as needed in patients with minor discomfort. Disease that is more serious is treated with PPI for periods or continuously and in some cases with a double dose. Adapted from Tidsskr Nor Laegeforen (112).

Proton pump inhibitor (PPI)

PPI are the treatment of choice in patients with GERD affecting the quality of life. They inhibit acid secretion in parietal cells irrespective of stimulus. The effect is best

for meal-induced reflux and it increases over days. Short-term use has not been associated with complications. A restrictive attitude to long term treatment is recommended, and the dose should be kept as low as effective (112).

The effect of PPI may be better in classical GERD than in EER

PPI treatment at low doses once daily will elevate gastric pH to 6 or higher, but only for short periods. Higher doses twice a day seem to have a better effect.

Histamine-2 receptor antagonists

Hitamine-2 receptor antagonists give quick symptom relief through reduction of acid production in the stomach.

Antacids

Antacids neutralizes the acid in the stomach. The effect comes quickly but will not stop further acid production.

Alginates

Alginates make a mechanical barrier that protects against the postprandial acid pocket. Studies show significantly better effect compared to placebo. There does not appear an additive effect of supplying PPI treatment with alginates (113).

Antireflux surgery

Fundoplication was described first time in 1955. Anti-reflux surgery may reduce esophageal acid reflux significantly, but any long-term effect has been difficult to predict (114). Most patients achieve and remain in remission five years after laparoscopic antireflux surgery (115).

Lifestyle intervention

As behavioral factors may trigger GER episodes, lifestyle intervention may improve the disease (78). Moderate physical exercise and high intake of fiber seem to reduce the risk but systematic review on life style interventions concluded that only weight loss in obesity and head-of-the-bed elevation were effective. However, several lifestyle interventions are believed to work and is often recommended; Lose weight if

obese, stop smoking, increase dietary fiber intake, avoid late evening meals, head-of-the-bed elevation if supine reflux (87). Data on elimination of food that can trigger reflux is not available.

4.5 The link between GER and the airways

A possible association between GERD and the upper airways, was first described more than 100 years ago. In 1903 Coffin suggested that "post-nasal catarrh" may be caused by hyperacidity and gases from the stomach (116).

It has been discussed for decades whether GERD, for some CRS-patients, can be a contributing factor. Holmes et al writes in the book, "The Nose", from 1950 about a possible connection between GERD and the upper airways (117), and the precise role of GERD in CRS has been discussed since that time without reaching a consensus. Montréal definition of GERD defines the status up to 2006 (1).

4.5.1 Pathophysiological mechanisms linking GER and the airways

Two different pathophysiological mechanisms have been suggested to explain how GERD may contribute to airway manifestation. A theory of direct cytotoxic effect of refluxate on the respiratory mucosa and a reflex theory suggesting a neural reflex from the esophagus to the upper airways via the autonomic nervous system (118, 119).

The theory of a direct toxic effect

This theory implies there is a direct toxic effect from the refluxate on the airway mucosa (Figure 9) (118).

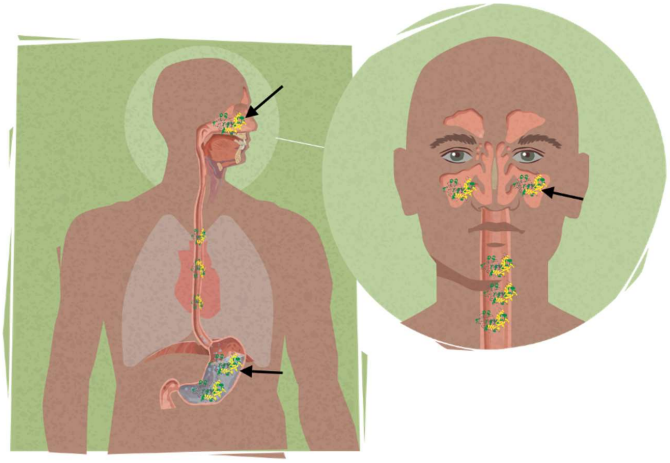

Figure 9. One theory is that the refluxate has a direct toxic effect on the extraesophageal mucosa. In this illustration, pepsin from the stomach has reached the sino-nasal area.

The mucosa outside the stomach has limited protective ability against gastric content. Thus, pepsin may induce inflammation in the respiratory tract with mucosal edema and impaired mucociliary function. In children, 24-hour pH monitoring has shown reflux to nasopharynx. Contencin and Narcy showed reflux above the cricopharyngeus which may support the existence of direct toxic effect of refluxate (120). The theory is further supported by detection of pepsin in saliva in pediatric patients with documented reflux and less pepsin in controls (110). Detection of pepsin in the middle ear of children with recurrent acute otitis media and/ or otitis media with effusions, is also in line with this theory (121).

The reflex theory

According to the reflex theory, there is a vago- vagal reflex from the esophagus to the airways via the autonomic nervous system. The existence of such a link is supported by studies made decades ago. Intra-esophageal acidic provocation did reduce airway flow and heart rate and increased pulmonary resistance in asthmatics (122, 123). Stimulation of the esophagus in guinea pigs with hydrochloric acid elicits inflammation in the trachea (124). As to CRS, the theory is that a neuronal induced

nasal mucosal edema consequently gives ostial obstruction (125). Our results, with no important difference in pepsin, in nasal secretions and saliva, between CRS-patients and controls and no significant difference in pepsin in nasal secretion or saliva samples between CRS-patients with proximal reflux or not, may be in line with this. Reservations have to be made due to the possible limited sensitivity and specificity of the Pep-test (126).

4.5.2 The link between GER and inflammation of the lungs

There is a high prevalence of GER in asthmatics, frequently without the classical symptoms of GERD. Pepsin has been detected in the lungs, and elevated level of pepsin from the lungs has correlated well to severe pulmonary symptoms (127)

In a trial with PPI treatment of patients having both asthma and GER, there was a reduction of nocturnal asthma (62). In another study, a subgroup of asthmatics with concomitant diagnosed GERD may have significant improvement from PPI treatment (128). However, trials on treatment of asthma with PPI are diverging, and currently there is no evidence to support a routine use of PPI in asthmatics.

4.5.3 The link between GER and chronic cough

Chronic cough may be initiated by aspiration of gastric content. Cough may also be due to distal esophageal stimulation, via a reflex mechanism (129). Although GERD is one of the most common causes of chronic cough, the diagnosing and treatment of GERD-related chronic cough can be challenging. Only a small proportion of these patients have the classical symptoms of GERD (130), but a high proportion have pepsin in saliva when tested immediately after an episode of cough (131).

4.5.4 The link between GER and laryngitis

When the gastric content ends up more proximally in the laryngopharynx, it is called laryngopharyngeal reflux (LPR). LPR is relatively common and is frequently associated by laryngeal symptoms. In 2000, Koufman described that at least 50% of patients with laryngeal and voice disorders, referred to a tertiary voice center had GERD (93). These patients did not necessarily have the typical symptoms of GERD

and they had less than 40% heartburn and 25% esophagitis (81). More recently, Wang et al found pepsin in vocal cord polyps, concluding that pepsin may be a risk factor in their formation (132). Though the connection between GERD and laryngitis is well established, there is no consensus on treatment procedures.

4.5.5 The link between GER and otitis media

A high incidence of pepsin in the middle ear cleft of children with otitis media with effusions (OME) led to speculations about a role of reflux in the pathophysiology of otitis media with effusion, and a role of anti-reflux therapy in patients with this diagnosis (133). Later, this was confirmed in a controlled trial of 509 of children with recurrent acute otitis media and/ or otitis media with effusions and a control group of 64 pediatric patients. They found a high incidence of pepsin in the middle ear cleft of children with recurrent acute otitis media and/ or otitis media with effusions, compared to a control group (134). As GERD has been implicated in the pathophysiology of otitis media with effusion, authors have concluded that there may be a role of anti-reflux therapy in patients with otitis media with effusion (121). Helicobacter Pylori (H. Pylori) has been detected in the middle ear in OME and may be a marker of reflux. Thus, the bacteria have a possible role in the pathogenesis of OME (135).

4.5.6 The link between GER and obstructive sleep apnea syndrome (OSAS)

OSAS is characterized by episodes of pharyngeal collapse during sleep, with ceasing airflow. These patients have a high incidence of nightly reflux. When treating with continuous positive airway pressure (CPAP) the reflux is reduced. The mechanisms of GER in OSAS are not fully understood, but obesity is predisposing for both diseases. Negative intrathoracic pressure and repetitive arousals may also play a role (136). As only a proportion of nocturnal GER episodes are associated with apneic events, and studies on the relationship between GER and OSAS are diverging, a causal relationship is not established (137).

4.5.7 The link between GER and dental erosions

Dental erosions is considered an established complication to GERD (1). Reported prevalence in patients with symptomatic GERD is between 17% and 68% (138) and a prevalence of 29% (139) is seen in European adults without the classical symptoms of GERD. As measured with impedance pH monitoring and endoscopy, the majority of patients with dental erosion had GERD (138).

4.5.8 The link between GER and CRS

Looking for a link between GER and CRS, it has been suggested that three criteria should be met.

1. Patients with CRS should have a higher prevalence of GER than healthy controls.
2. Biologically plausible pathophysiological mechanism should explain the link (Figure 10).
3. If GER is a contributing factor to CRS, clinical manifestation of suspected GERD-related CRS should respond to anti-reflux therapy (119).

Our study on nose- and sinus-related quality of life in GERD, showed that the former was reduced in CRS-patients compared to heathy controls, as measured with SNOT-20. The study supported a coexistence of GERD and CRS (140). Contencin and Narcy were the first to demonstrate acid reflux in children with chronic rhinopharyngitis (120). Patients with a refractory CRS have more symptoms of GERD (141), and in CRS-patients undergoing FESS, GERD was a predictor of poor outcome of the surgery (142). In pediatric patients, Phipps et al found that 63 % of those suffering from CRS had GER (143). A questionnaire-based trial found nocturnal GERD to be a risk factor of developing rhinitis and rhinosinusitis (144).

Adult patients with refractory CRS have more reflux compared to a control group as measured with pH-monitoring (145). Frequent episodes of proximal reflux found by 24-hour multichannel intraluminal pH-monitoring in CRS-patients compared to normal data indicates that reflux may reach the upper airways (146). Detection of

nasal pepsin in CRS-patients has been regarded as a proof for an association between GERD and CRS (79). These findings support that a direct toxic effect of refluxate may be a pathophysiological mechanism in CRS. Our controlled trial using Peptest for this purpose did not reproduce these results, concluding that this may be due to low specificity of the test (126).

As lifestyle intervention may increase the tension in LES and reduce the occurrence of TLESR, they may also improve GERD-related CRS. We have not seen that this aspect investigated. The effect of anti-reflux therapy in CRS is better documented in children than adults. Bothwell et al. found that, in children with CRS who were candidates for surgery, 89 % experienced symptom relief after anti-reflux therapy and were able to avoid an operation. They concluded that GERD should be considered and treated medically before surgery in children (147). Phipps et al. found that 15 of 19 children diagnosed with both GERD and CRS improved regarding CRS-symptoms after medical reflux therapy (143).

DiBaise found retrospectively, an improvement of sinus symptoms in two thirds of adult patients with clinically refractory CRS with GERD after PPI or anti-reflux surgery. Only the patients who had abnormal esophageal pH-monitoring had improvement of their sinus symptoms (148). These results were not reproduced in a later controlled pilot trial (149).

Repeated treatment studies have recommended higher doses and longer period of treatment when treating EER, CRS included, before evaluation of effect (81, 150, 151). PPI b.i.d. when treating EER is recommended considered in some patients after thorough examination (105).

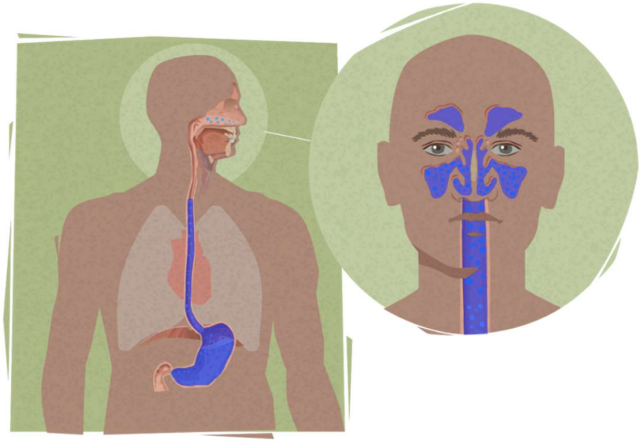

Figure 10. This illustration of GER in CRS indicates that the refluxate provides an inflammation of the sino-nasal mucosa. The inflammation may be induced by a direct toxic effect or by a reflex from esophagus to the airways.

5. Aims of this thesis

5.1 General aims:

The main aim of this research was to obtain a better understanding of the role of GER in CRS and to get a final answer to the question: Is there an association between GERD and CRS?

5.2 Specific aim Paper I

We wanted to investigate the sino- and nasal-quality of life in patients with gastroesophageal reflux disease (GERD) compared to a control population.

5.3 Specific aim Paper II

We wanted to monitor the occurrence of reflux in CRS-patients by using with the most reliable method for this purpose. We considered 24-hour esophageal multichannel impedance pH-monitoring to be the best choice.

5.4 Specific aim Paper III

We wanted to evaluate the presence and level of pepsin in sputum and nasal secretion in CRS-patients compared to age and gender matched healthy controls and consider if Peptest is a valid tool in the monitoring of GER in CRS.

6. Subjects and methods

6.1 Study design and subjects

This thesis is based on three prospective controlled studies. In the first, consecutive adult patients referred to Gastro Nova, a specialist clinic in gastroenterology, were included if fulfilling the criteria of having GERD with esophagitis. We investigated the nose- and sinus-related quality of life in these patients by using SNOT-20. The patients filled out the form in the gastro outpatient clinic immediately after gastroscopy.

In study number two and three, consecutive adult patients with CRS referred to the ENT department at Stavanger University Hospital, were included after informed consent. In study number two, the CRS-patients went through a 24-hour impedance pH-monitoring. The results were compared with healthy controls from an existing European database collected by French and Belgian clinicians (103). In the third study, we analyzed and compared pepsin in nasal secretions and saliva in CRS-patients and age and gender matched healthy controls.

Ethics and approval

The study was conducted according to the guidelines given in the Helsinki declaration. The Regional Norwegian Committee for Medical and Health Research Ethics (REK Vest 2010/2030) approved all procedures. All participants were included after written informed consent.

Nose and sinus-related quality of life in GERD (Paper I)

Patients with suspected GERD, referred to Gastro Nova, a specialist clinic in gastroenterology, were included in the study after positive gastroscopy. The patients fulfilled the criteria for GERD (1) and had a positive gastroscopy. The esophagitis was staged according to the Los Angeles (LA) classification; Grade A: mucosal breaks < 5 mm long and do not extend between the tops of two mucosal folds, grade

B: mucosal breaks > 5 mm long and do not extend between the tops of two mucosal folds, grade C: mucosal breaks that is continuous between the tops of two or more mucosal folds but which involves less than 75% of the circumference, grade D mucosal break which involves at least 75% of the esophageal circumference (97). This is the most widely used system to endoscopically grade GERD (152).

Seventy-seven patients were included, 44 men and 33 women, median age 50 years, ranging from 19 to 83. Fifty-two were classified as LA grade A, ten as grade B and 15 as grade C.

480 randomly selected teachers were included as healthy controls, 183 men and 297 women, median age 43 years, ranging from 20 to 75 (Table 2).

Table 2. Demographic data of the patients and healthy controls in the SNOT-20 study.

	GERD-patients, N=77	Healthy control subjects, N=480
Age, median (IQR), years	50 (39-61)	43 (35-54)
Sex, Women/ Men	33/45	297/183

IQR= Interquartile Range

The enrolment of patients took place in the period 2008- 2009. The enrolment of healthy controls took place in the same period.

Impedance pH-monitoring (Paper II)

Consecutive patients with CRS according to the criteria given by EPOS 2012, above 18 years of age who were referred to the ENT department, Stavanger University Hospital were invited to participate. GERD with ongoing treatment for reflux was an exclusion criterion. Symptoms of reflux or prior sinus surgery were not. Twenty-one women and 25 men were included, median age 47 years, ranging from 23 to 73 (Table 3). The enrolment of patients took place in the period 2013- 2015.

The control subjects were participants in a European study published in 2013, 22 men and 23 women, median age 47 ranging from 18 to 78 (Table 3).

Table 3. Demographic data of the patients and healthy controls in the pH-impedance trial

	CRS-patients, N=46	Healthy control subjects, N=45
Age, median (IQR), years	47 (40-58)	47 (35-58)
Sex, Women/ Men	21/25	22/23
BMI, median (IQR)	25.1 (23.5-26.1)	24.0 (21.9-25.0)

IQR= Interquartile Range BMI= Body Mass Index

All patients were informed and examined by the same investigator. The patients completed 24-h impedance pH-monitoring, with reliable recordings for 18-25 hours.

Pepsin measuring (Paper III)

Consecutive patients with CRS above 18 years, referred to the ENT department, Stavanger University Hospital were invited to participate. Sixty-two patients were included, 33 men and 29 women. Age and gender matched healthy controls were recruited among patient's friends, neighbors, colleagues, at different jobs and among the investigator`s friends, family, colleagues and parents of children's associates. The enrolment of patients and healthy controls took place in the period 2013- 2017. Median age in patients and healthy controls was 46 (ranging 22-73) and 46.5 (ranging 21-71) respectively (Table 4).

Table 4. Demographic data of the patients and healthy controls in the pepsin study

	CRS-patients, N=62	Healthy control subjects, N=62
Age, median (IQR), years	46 (36-56)	46.5 (36-54)
Sex, Women/ Men	29/ 33	29/ 33
BMI, median (IQR)	25.1 (23.5-26.9)	25.9 (23.3-28.4)

IQR= Interquartile Range BMI= Body Mass Index

Exclusion criteria was GERD with anti-reflux medication on a daily basis. Symptoms of reflux were not an exclusion criteria per se. Nasal corticosteroids were not allowed the day of investigation.

6.2 Methods

6.2.1 Questionnaires

All the questionnaires used are presented in the appendix

20-item Sino-Nasal Outcome Test (SNOT-20)

SNOT-20 was used in all the trials in this thesis. It is a validated, self-administered quality of life instrument specific for symptoms of rhinosinusitis and sensitive to clinical changes. It describes the health burden of rhinosinusitis by measuring; physical problems, functional limitations and emotional consequences of CRS by asking the participants to score 20 key symptoms. These are: the need to blow the nose, sneezing, runny nose, cough, postnasal discharge, thick nasal discharge, ear fullness, dizziness, ear pain, facial pain/ pressure, difficult falling asleep, waking up at night and difficulty falling asleep. With reference to symptoms the two last weeks, the participants scored each symptom from 0-5, giving a summery score, the total SNOT-20 score (52). A 22 items questionnaire, SNOT-22 (53) is available in English, but not validated in Norwegian, and thus not used in our studies.

Visual Analogue Scales (VAS-CRS)

VAS-CRS is a psychometric response scale used to measure subjective characteristics of sino-nasal symptoms. It includes the rating of twelve symptoms related to nose and sinuses as well as obstructive sleep disorders on a 100 mm VAS with endpoints "never" (0) and "always" (100). Each subject was asked to grade each symptom and condition by frequency. VAS 0-3 is defined as mild disease, >3-7 as moderate disease and VAS >7 as severe rhinosinusitis affecting the patient´s quality of life defined in EPOS 2012 (2).

Gastro-esophageal Reflux Disease Questionnaire (GerdQ)

GerdQ was used to record classical symptoms of GERD. It is a six-item validated, self-assessed questionnaire regarding frequency of symptoms typical for reflux the last seven days (90, 91). It is developed from the 22-item questionnaire Reflux Disease Questionnaire (RDQ), a self-assessed questionnaire developed to diagnose and evaluate GERD in general practice (89). It is valid as a diagnostic tool for GERD with reduced health care costs compared to endoscopic approach. In a trial from 2012, the authors found that by using the GerdQ, they reduced health care costs (90). GerdQ may not capture atypical symptoms of GERD. Scores above eight indicate reflux disease.

6.2.2 Twenty-four-houre esophageal impedance pH-monitoring

Twenty-four-hour combined esophageal pH- and impedance-monitoring is regarded the gold standard for investigation of GERD (101). As reflux is best detected by impedance and its acidity best characterized by pH-monitoring, we chose this method in our trial.

Intraluminal impedance monitoring allows detection of gastro esophageal-reflux based on changes in resistance to electrical current flow between two electrodes, as liquid and/ or gas bolus moves between them. A 50 % drop in impedance from baseline, seen as a retrograde move along the catheter starting distally characterizes an episode of reflux. As the refluxate flows back into the stomach, a rise in impedance develops as air has low electrical conductivity. The change in impedance

during an episode of reflux leaves a typical measuring pattern (Chapter 4.4.4, Figure 7a-7d).

The number of distal reflux episodes and the number of reflux episodes with proximal extent, defined as reaching the impedance electrodes 15 cm proximal to the LES, were recorded. Bolus exposure was calculated as percent of total recorded time. We differentiated between reflux when recumbent or upright, as well as acidic (pH<4) or non-acidic (pH>4) reflux.

The impedance pH-catheter was positioned trans-nasally into the esophagus with the proximal pH-electrode located five cm above the LES and one in the stomach. The impedance electrodes were positioned 2, 4, 6, 8, 10, 14, 16 and 18 cm above the LES. As impedance was monitored between two electrodes, it was monitored at six different levels (Figure 11). The participants had an overnight feast before undergoing esophageal manometry to localize the level of the upper and lower esophageal sphincters (UES/LES).

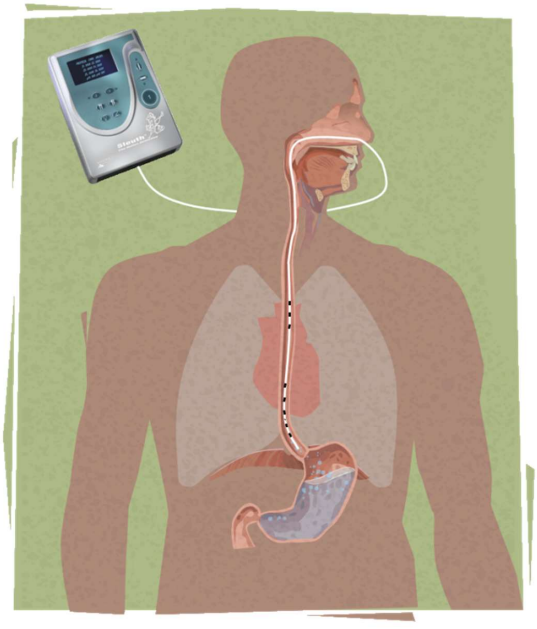

Figure 11. 24-h impedance-pH monitoring, showing the catheter in the esophagus with eight impedance electrodes giving six measuring points and an external device for registration.

Patients completed the 24-h combined pH-impedance monitoring, with recordings for 18 - 26 hours. The impedance pH-catheter ZAN-BG-44 (Sandhill Scientific, Inc; Highland Ranch, CO, USA) was used.

The participants were encouraged to maintain normal daily activities and take meals as usual during the recording. They were asked not to drink beverages with a pH<4 between meals. A data logger (Sleuth, Sandhill Scientific Inc.) (Figure 11) was used to record meal times, changes in posture and the occurrence of the following specific symptoms: regurgitation, chest pain, cough and the need to clear the throat. We scored the symptom-reflux time in line with the Symptom Index (SI) (153) and Symptom Association Probability (SAP) (154).

The healthy controls underwent a similar study protocol for 24-h esophageal impedance pH-monitoring (103).

The analysis was performed with BioView version 5.4.3 (Sandhill Scientific Inc.). They were done automatically with the Autoscan function of BioView, and then hand edited by two investigators. Each control person was also analyzed manually by the same two investigators.

Measuring pharyngeal reflux has been attempted, but the investigational techniques are difficult and poorly reproducible (103). Thus, such recordings were not included in the present study.

6.2.3 Peptest

As twenty-four-hour impedance pH-monitoring is a resource consuming procedure and promising results on pepsin monitoring as an alternative diagnostic procedure of GERD appeared, we wanted to examine this in EER.

We used an enzyme immunoassay, Peptest® (RD Biomed Ltd, Cottingham, UK), recommended for detection of pepsin in saliva. This non-invasive test is based on a lateral flow device technology. It contains two different monoclonal antibodies to human pepsin and is specific to pepsin A, the isoform secreted only in the stomach

(71, 131). A control band on the device indicates a correctly performed test and a second line indicates presence of pepsin in the sample, defining a positive or negative test. The intensity of this line is proportional to the quantity of pepsin, and by using a reader, LFDR101, the concentration of pepsin in ng/mL is obtained (131). Both the sensitivity and specificity of Peptest has been estimated to be 87 % (106) but recent literature suggests the sensitivity to be 78,6 % and the specificity to be 65% (155). The lower limit for detection of pepsin with Peptest is 16ng/ mL given by the manufacturer (131). To the best of our knowledge, Peptest has not previously been used on nasal secretions.

CRS-patients and healthy controls delivered three tubes with saliva and three tubes with nasal secretions (roughly 1 mL in each) for pepsin analysis. We strictly followed the recommendations from the manufacturers; “Sample one should be taken in an upright position within 15 minutes of waking up from overnight sleep before eating or brushing teeth. The second sample should be taken one hour after the main meal of the day and the third sample should be taken one hour following the next main meal of the day” (155). This gave a total of three saliva samples and three samples of nasal secretions per subject. Subjects without rhinorrhea or not having enough nasal secretions to create samples from the nose, used 0.5 ml sterile water for irrigation of each nostril. They were asked to inform whether they used sterile water in the nose or not. Unfortunately, this information is missing in a large proportion of both patients and healthy controls. However, roughly 45 % of the patients did use water for irrigation, slightly more of the controls (Table 5).

Table 5. Percent of patients and controls that used sterile water, 0.5 ml in each nostril before collecting the nasal sample.

	Pas A1 %	Pas A2%	Pas A3%	Con A1%	Con A2%	Con A3%
no	13,5	12,2	14,9	10,8	16,2	17,6
yes	45,9	45,9	35,1	56,8	50,0	48,6
missing	40,6	40,5	50,0	32,4	33,8	33,8

The tubes for investigation contained 0.5 mL of 0.01 M citric acid for conservation, and the participants stored the samples in a refrigerator until analysis. As recommended the samples were analyzed within seven days after they had been collected (106).

Each sample was centrifuged at 5000 rpm for five minutes (Figure 12 a), 80 µl of the supernatant was extracted, and 240 µL buffers were added. This was vortex-mixed (Figure 12 b) for ten seconds before 80 µL was transferred to the pit of the Peptest device (Figure 12 c). The test was read as positive or negative after 15 minutes and quantification done by the LFDR reader (Figure 12 d).

Figure 12 a. The centrifuge used for centrifuging the six samples per subject.

Figure 12 b. Vortex mixer.

Figure 12 c. Eighty µL solution transferred to the Peptest device.

Figure 12 d. The LFDR-reader used for quantification of pepsin detected with the Peptest device.

In samples with no supernatant layer, 500 µL buffer was added, and the sample was Fortex mixed for ten second and centrifuged a second time at 5000 rpm for five minutes. If a supernatant layer appeared, the procedure as described was performed, if not, another 500 µL was added and centrifuging repeated as recommended by the manufacturer (personal message from Dettmar and Woodcokk, RD Biomed Limited).

The same investigator performed all the tests on both CRS-patients and healthy controls.

6.3 Statistics

Descriptive data are reported as median with inter-quartile range, mean with standard deviations and counts with percentages, as appropriate. As data in these studies generally were not normally distributed, non-parametric methods were used. The analyses were done using SPSS (Statistical Package for Social Sciences, Chicago, USA), SPSS ver. 23 (Paper I and II) and SPSS ver. 24 (Paper III).

Prior to the studies, we performed sample size calculations, in order to decide the minimum sample size needed to recognize a given difference between groups. In trial one and two, we had an already collected number of healthy control subjects included in this calculation.

In the analyses of subgroups, there are lower number of participants, hence the statistical power of these analyses is lower.

In trial 1 and 2, the data were not normally distributed, and we used Mann-Whitney U test for differences between patients and controls.

The data in trial 3 were also not normally distributed. As the data were collected as age and gender matched, we used the paired samples Wilcoxon for continuous data and McNemars test for categorical data. To calculate correlation between variables, we used Spearman's Rank Order Correlation. In all hypothesis tests, a p value less than 0.05 were considered to be statistically significant.

7. Summary of results

7.1 Results Paper I

Patients with GERD had significantly higher total SNOT-20 scores compared to a control group. Median total SNOT-20 score in patients was 22.1 and in healthy controls 5.0 ($p<0.005$). Hence, they had a reduced nose- and sinus-related quality of life compared to the control group. We also found significant higher SNOT-20 scores in the patient group when omitting the possible sleep-related questions in the questionnaire. Accordingly, this study indicated that there may be an association between GERD and CRS.

7.2 Results Paper II

CRS-patients had significantly higher incidence of abnormal gastroesophageal reflux compared with asymptomatic controls. The esophageal acid exposure in percent of time in patients compared to healthy controls was 7.3 and 1.4 respectively ($p<0.03$). The number of events with proximal reflux was higher in patients compared to controls, median 34 and 3 respectively ($p<0.0005$). The results of this study suggested that GER might be a causative or contributing factor of CRS.

We found evidence of more GER in all patients compared to controls as measured with both acid exposure in percent of time, number of reflux episodes, bolus exposure in percent of time and especially large difference between the groups in proximal extent of the refluxate.

7.3 Results Paper III

As measured by Peptest, we found frequently positive Peptest and high levels of pepsin in saliva and nasal secretions in CRS-patients and healthy controls. However, we did not find more pepsin in saliva or nasal secretions in CRS- patients than in

healthy controls, nor in those with high GerdQ scores or verified proximal reflux. There was no correlation between pepsin and GER as monitored with 24-h impedance pH-monitoring. We found a high degree of variation of the concentrations, both in CRS-patients and controls.

This indicated a limited validity of the Peptest as screening tool for GER in CRS.

8. Discussion

8.1 Study design

The three papers in this thesis are based on three different studies where a prospective control design was used, all having different approaches to the issues of the thesis. The design can give important contribution in evaluating a hypothesis, but when interpreting the data, caution must be taken, particularly in establishing a relationship between cause and effect.

8.2 Selection of patients and control subjects

We have strongly focused on the selection of participants to ensure external validity. All patients and controls were included after information and written consent.

8.2.1 Patients

The patients in study number one were included after fulfilling the criteria of having GERD according to classical symptoms and pathological gastroscopy findings. We recruited patients from Gastro Nova, a specialist clinic in gastroenterology in Stavanger, Norway. As all invited patients accepted to participate, there should not be a selection bias. Only patients with a positive diagnosis of reflux esophagitis by gastroscopy were included.

All patients in study number two and three were included from the ENT department, Stavanger University Hospital. They had CRS according to EPOS 2012 (2). We did not distinguish between CRSwNP and CRSsNP, and prior sinus surgery was not an exclusion criterion. Only ten of the CRS-patients declined. The reasons were long distance to hospital and unwillingness to swallow the probe.

8.2.2 Healthy control subjects

The control subjects in all the studies in this thesis were at least 18 years old.

In study number one, the controls were all schoolteachers at different schools in Vest Agder County, Norway, included after information and written consent. A co-author visited the schools, gave instructions about how to complete the questionnaires and collected them afterwards. By this, nearly all present teachers participated, and no forms were lost.

Teachers have an average level of education, an average level of income, and a reasonably equal distribution of males/females in the Norwegian society. They were therefore considered applicable as controls in this these studies.

The control population in study number two was an already existing material used by European clinicians as the normal values for 24-hour impedance pH-monitoring.

The control population in the third case-control study was enrolled in the Stavanger area in the same period as the patients were recruited. To ensure external validity, they were included from different arenas. They were teachers, store employees, from various offices and among the PhD candidate and patient's friends and colleagues. The healthy controls were age and gender matched to the patients in the study.

8.3 SNOT-20

We used the 20-item questionnaire "sino-nasal outcome test-20" (SNOT-20) (52) and not the more recent SNOT-22 (53), as the former was validated in Norwegian, and the latter was not at the time of investigation. The differences between the two questionnaires are questions about nasal blockage and the loss of sense of taste and smell in the latter. It is hard to imagine that the lack of these questions may have made any significant effect on the outcome of the present investigations. However, as there is a significant association between disturbed sleep and GERD (156), sleep quality could be a potential bias (157). To exclude this possible source of error, we also compared the groups omitting the sleep-related questions. The difference between patients with GERD and the healthy controls was still significant with $p<0,001$ using SPSS and the Mann-Whitney U test.

As there was a preponderance of women in the control group (61.9%) and men (57.7%) among the patients, we checked if differences in sex could explain the differences between the two groups. We did not find any statistically significant differences in SNOT-20 score between men and women, not amongst patients, nor amongst controls.

As the age was lower in the control group compared to the patient group, median age 43 and 50 respectively, it may be speculated whether this may affect the SNOT-20 outcome. The differences in age were small, but due to the large sample sizes, they were significant ($p=0.04$). When separating the patients and controls in two groups, above and below 50 years, we did however not find any significant differences in total SNOT-20 score between the groups.

We also separated the patients and controls in other age groups (above and below 40 years and above and below 60 years) without finding significant difference in total SNOT-20 between the ages. Accordingly, there is no indication of any age effect in the SNOT-20 score in this study.

Seventy-seven out of 100 patients participating in the study had endoscopic findings indicating reflux esophagitis. The other 23 patients had not and were not included in the analysis. There was no difference in SNOT-20 score between these groups. This may be explained by the fact that the gastroscopy will only capture the patients with GERD and visible lesions (158). We included patients after positive gastroscopy, and we may have excluded patients with reflux, but without evidence of mucosal damage.

Our results are supported by another study using SNOT-20 to compare symptoms of post-nasal drip (PND) with 24-hour esophageal, nasal and laryngeal pH-monitoring. They found PND to be associated with reflux (159).

Generally, history, pH-monitoring and / or gastroscopy may establish the diagnosis of GERD. In the SNOT-20-study, the patients were included based on symptoms of GERD and the findings of mucosal breaks at gastroscopy. This combination has a

high specificity of 97% (152, 158). Accordingly, it is reasonable to believe that the patient included really had GERD and thus, that we have given a description of the sino-nasal quality of life in a relevant group of patients and compared them with a relevant group of healthy controls.

8.4 Twenty-four-hour esophageal impedance pH-monitoring

It has been debated to what extent automatic analysis of impedance data with software such as Autoscan® (Sandhill Scientific Inc.) is reliable and comparable to manual analyses. A software program must be able to distinguish between impedance changes due to real reflux and changes due to non-reflux phenomena such as swallowing, artifacts and random changes in intra-esophageal impedance. The software seems to have improved over the years in this respect as there is only minor variability between hand-editing with Autoscan versus Autoscan alone. Still, today, the former is recommended (160). Zerbib et al. found also a small inter- observer discrepancy of proximal events between different investigators. This was due to both missed events and misdiagnosed events, based on different interpretations of impedance drops within the esophagus (103). Never the less, the overall correspondence between manual and automatic analyses was good (161).

To reduce the possible bias because of different investigators analyzing the two groups, patients and controls, we combined automatic and manual analysis of patients´ recordings and agreed about the evaluation of each (Figure 13). We removed a percentage of proximal episodes that we could not accept as indicative of reflux but did not inspect the entire recording for possible missed events. This might result in underestimation of number of episodes of proximal reflux.

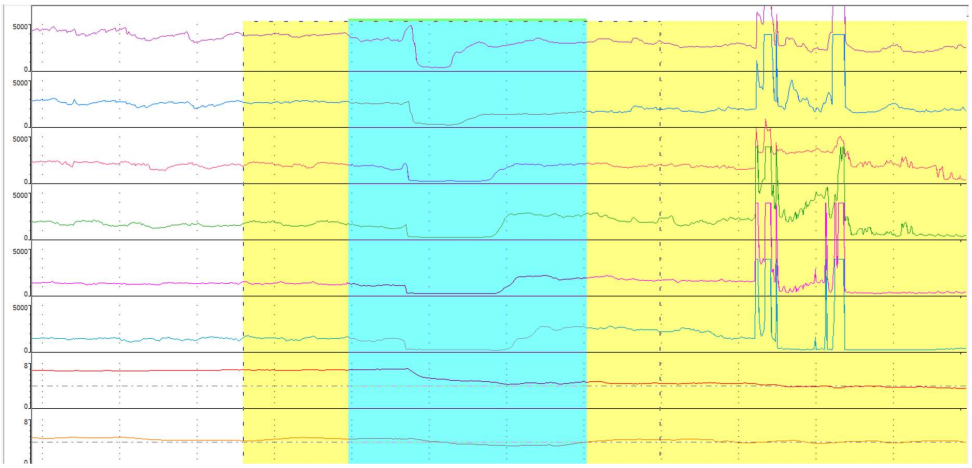

Figure 13. Example of an episode of proximal reflux as identified with impedance, a fall in impedance starting at the distal measuring point and the increase of impedance starting proximal as the refluxate is returning to the stomach.

Furthermore, one of the two scientists that did the analysis had previously done similar analyses with the French group that handled the control data. This is likely to have increased the reliability of the interpretation.

There has been an assumption that the prevalence of GERD in North America is higher than in Europe, and furthermore that physiological reflux may be more pronounced. These geographical variations have been explained by cultural and dietary differences (Shay, Tutuian et al. 2004), and were the background for establishing a European database for normal pH-impedance values. However, the total number of reflux events detected by impedance in the European and the North American studies, were very similar and gave no support to the theory of differences between the populations (103, 104). The European control material used in this trial is used all over Europe for similar investigations. Hence, lacking our own normal data, this was the best way for us to perform a controlled study. Professor Frank Zerbib at the University of Bordeaux generously offered us full access to the entire database of his institution.

We have focused on impedancemetry, more than pH-monitoring in this study, since we took particular interest in proximal extent of reflux. As we found high incidence of non-acid or weakly acidic exposure as measured with pH-impedance, and especially abnormally high number of proximal reflux episodes, it was interesting to know if that observation was in line with traditional pH-measurements. All the patients with pathological acid exposure, defined as having acid exposure time (AET) (time below pH 4.0), AET > 7,3 % of recording time, did also have abnormal proximal reflux defined as number of episodes > 15.5/ 24 hours (Figure 14 a). Upper limit of normal based on the control group from Bordeaux is higher than the Lyon Consensus values (> 6% abnormal, 4- 6% borderline). The results of acid exposure combined with distal reflux estimated as number of distal impedance events is shown in Figure 14 b. Bolus exposure in percent of time combined with acid exposure is shown in Figure 14 c.

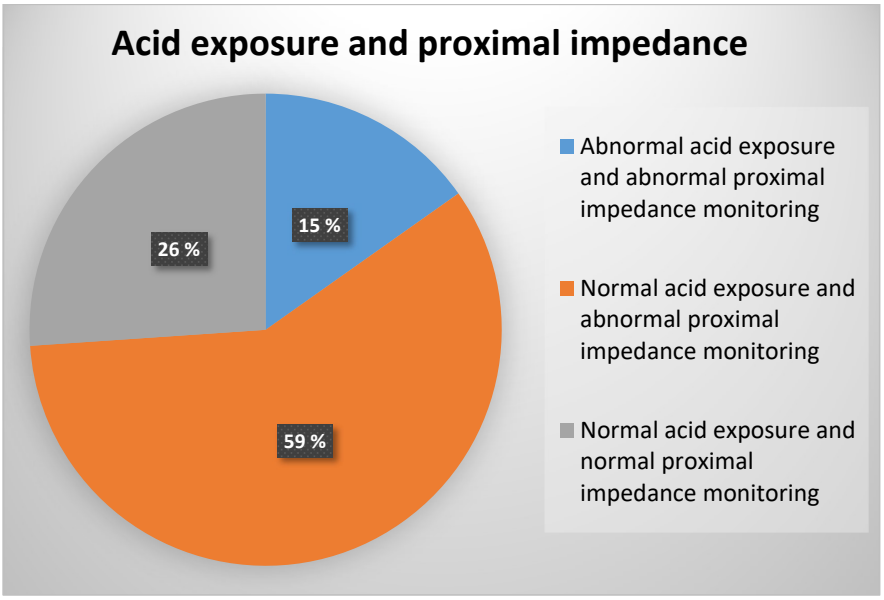

Figure 14 a. Characteristics of the patients with proximal reflux as measured with 24-hour impedance pH-monitoring. All patients with pathological acid exposure, as measured in percent of time, had abnormal proximal extent of refluxate.

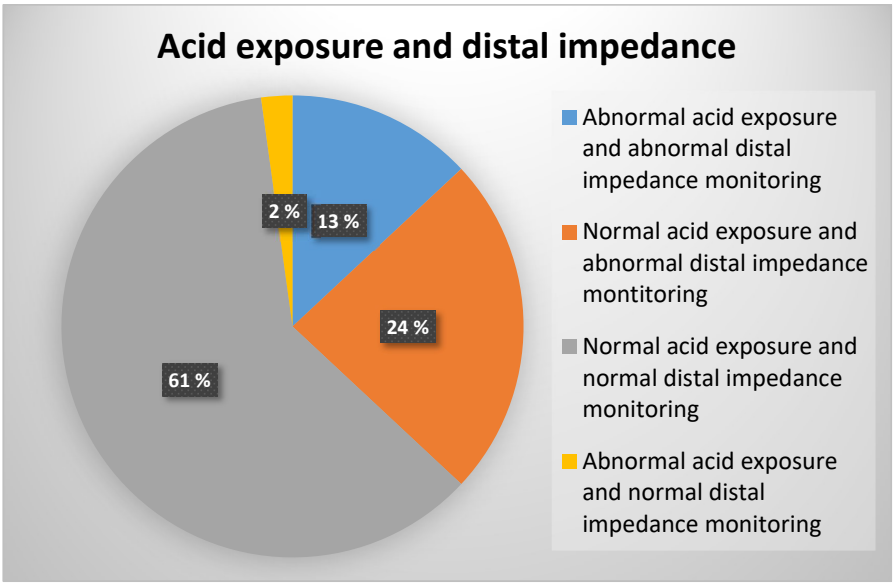

Figure 14 b. Results of 24-hour impedance pH-monitoring for the distal esophagus. Distal impedance results are based on number of episodes, and acid exposure are based on percent of time.

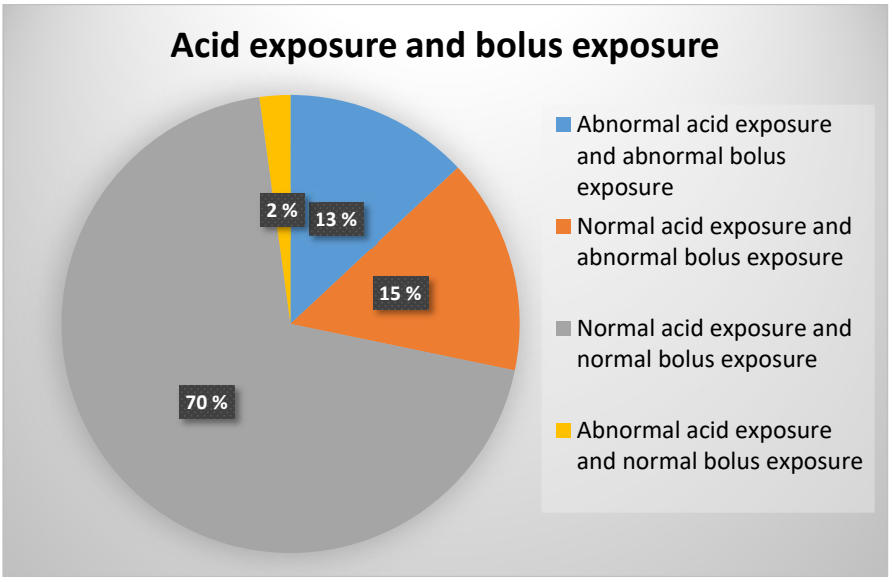

Figure 14 c. Bolus exposure and acid exposure, percent of time as measured with 24-hour impedance pH-monitoring.

If defining pathological acid exposure as AET > 6%, according to the Lyon Consensus (162) there is a higher number of patients included, and more if considering all patients with AET > 4%. Seven patients had AET >7.3% and ten had AET > 6% (additional three patients had AET between 4% and 6%). All except one patient with AET> 4%, had number of proximal reflux episodes of at least 22. As the 95th percentile in the European normal data from 2005 is higher than in our control material, it is interesting to know how many of our patients had an abnormal proximal reflux with these data as the reference. We had 22 patients with a number of proximal reflux episodes of 30 or higher. This is lower than the 34 patients we first measured as having abnormal proximal extent of the refluxate, but still a very high percentage of patients. When defining abnormal AET as > 6% of time and abnormal proximal reflux as > 30 episodes, the difference between the two is less. Abnormal distal acid exposure has traditionally been regarded as higher evidence of GER compared to number of reflux episodes as measured with multichannel impedance monitoring, and the proximal extent of impedance events as the least. Abnormal time association of symptom and reflux time (Symptom Association Probability=SAP) has been thought to provide the ultimate proof of GERD (Figure 15 and 16). The evaluation of SAP as parameter is more appropriate in investigation of classical reflux symptoms compared to investigation of EER, as it may be more difficult to state exact time of symptoms in the latter.

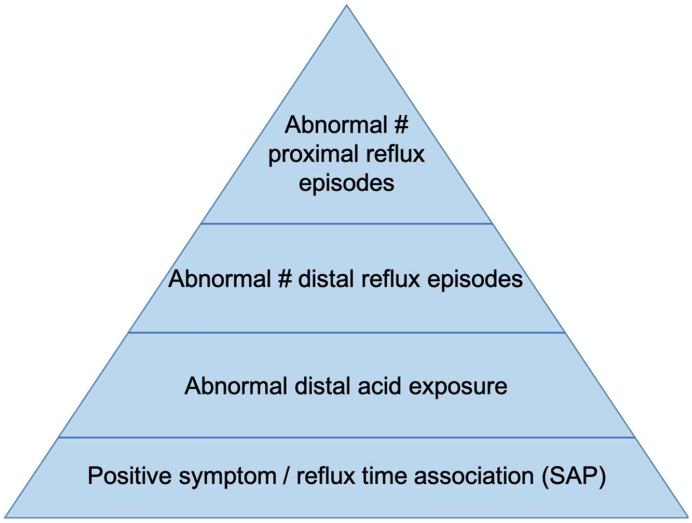

Figure 15. Evidence of GER is highest at the bottom of this figure. Increasing evidence of GER: Abnormal number of proximal reflux events, abnormal number of distal reflux events, abnormal distal acid exposure and SAP. SAP at the bottom of this figure has highest evidence of GER and abnormal proximal reflux, at the top, has the lowest.

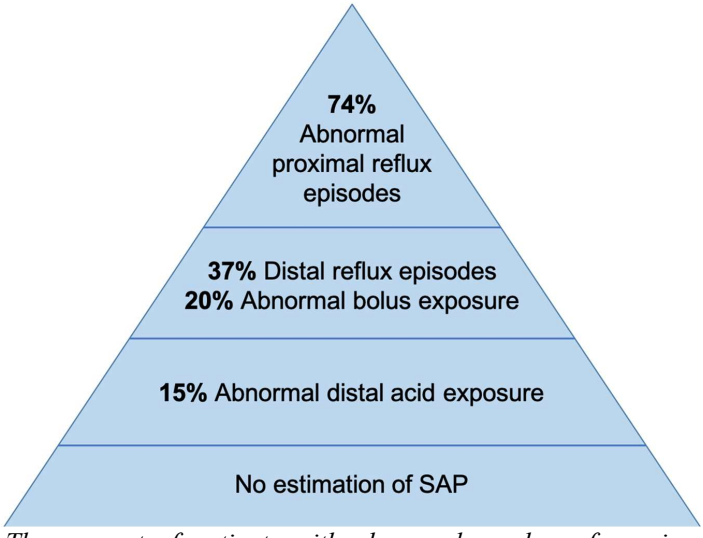

Figure 16. The percent of patients with abnormal number of proximal extents of refluxate, abnormal number of reflux episodes, abnormal bolus exposure and abnormal distal acid exposure as measured by pH impedance monitoring in our trial. Due to atypical chronic symptoms, we could not calculate the SAP (symptoms association probability)

In the diagnosis of EER, the proximal extent, with lowest evidence may be the most interesting parameter to investigate. This is due to speculations of the origin of the airway symptoms in EER. As the upper esophagus has nerve endings more superficial compared to the distal part (163, 164), a proximal extent of refluxate may have influence on more nerve endings and may more easily induce a reflex inflammation to the airways. The occurrence of proximal extent of refluxate might also support the theory that the refluxate has a direct toxic effect on the extraesophageal mucosa.

8.5 Peptest in evaluation of pepsin in saliva and nasal secretion

The same investigator handled all samples from all subjects and performed all the analysis with Peptest to ensure quality at all levels of the investigation. The investigator knew whether a test belonged to a patient or a healthy control. This lack of blinding may in principle be considered as a weakness of the study. On the other hand, it is difficult to imagine how this may have affected the outcome of the investigation.

The high number of positive Peptests, both in patients and controls, supports a high incidence of physiological, postprandial reflux. As we know that everybody has small amounts of GER, especially after meals, and most often without having symptoms from the esophagus (1), it is relevant to question if this still may give symptoms from the airways. Positive Peptests, both in those with and without CRS, may indicate differences in sensitivity to refluxate, so that a certain level of pepsin might create disease in some people, but not in others (68). This includes the upper airways. If there are differences in vulnerability to pepsin, pepsin might contribute to the development of CRS in certain predisposed individuals, but not in others.

The technical sensitivity of Peptest is confirmed to be high (106). However, the correct timing of collecting representative test samples represent a challenge, as

pepsin measurement in saliva is most reliable when collected at time of symptoms (165). Hence, Peptest gives snapshot information about the presence or absence of pepsin in saliva. In EER, the recommendation of obtaining the saliva samples soon after a reflux event (110) is difficult to accomplish practically. As reflux appears mostly postprandial, and corresponding symptoms of GERD are most common one to two hours after meals (166), we followed the recommendations from the manufacturer and tested post-prandial and immediately after waking up in the morning (to cover the nocturnal period) (155). Hence, any episodes of reflux at other times of the day may not have been detected.

Peptest specificity appears to be lower than previously estimated (106, 108). It can be speculated whether the characteristics of the test may have changed over time. The main reason for these speculations is the surprisingly high incidence of positive Peptest and high concentration of pepsin in samples from both healthy controls and CRS-patients. Differences in handling procedures of samples from patients and controls cannot explain these results. The number of days between collected sample and performed analysis was almost similar between the two groups, the median in CRS-patients was 3.0 days and in controls 3.5, both well within the recommendations of the manufacturer.

If a supernatant layer appeared, the procedure as described was performed. If not, 500 µL migration buffer was added and centrifugation repeated, as recommended by the manufacturer. As adding extra buffer gave a significant extra volume, and therefore theoretically could have given a lower concentration of pepsin, this was systematically evaluated and corrected for, again as recommended by the manufacturer, by multiplication of the concentration with 1.5, and 2.0 if buffer was added twice.

The present study raises important questions about the validity of Peptest as a screening method for extra-esophageal reflux. We strictly followed the recommendations from the manufacturers and found high levels of pepsin in both CRS-patients and healthy controls, with significantly higher levels of pepsin in saliva

in controls compared to patients. If Peptest were a reliable method for pepsin detection in order to diagnose EER, we would also have expected to find more tests that were positive in patients with abnormal proximal reflux as evaluated by 24-h impedance pH-monitoring compared to those without. We did not. Furthermore, we would also have expected to find fewer positive tests and lower concentrations of pepsin in healthy controls.

The significantly higher concentration of pepsin in the saliva of healthy controls compared to patients could be explained by the fact that reflux may induce hypersalivation (72). The hypersalivation may dilute the saliva (73), and the concentration of pepsin particularly in patients and thus, act as a confounder in the study.

As there was no correlation between Peptest results and GerdQ scores, or Peptest and 24-hour impedance pH-monitoring results, Peptest could not distinguish between physiological reflux and truly normal GER in our study.

Another research group has recently performed nasal and salivary pepsin measuring with Peptest on patients suffering from non-allergic rhinitis. All patients had positive Peptests, most frequently in the postprandial samples (70.79 %). They found a clear association between non-allergic rhinitis and GERD based on high pepsin concentrations and concluded that Peptest had a higher specificity compared to RDQ (167). In contrast to our study, this study was not controlled.

Recently, Sifrim`s group published a trial aiming to confirm previous data on high specificity of Peptest in healthy control subjects and patients with heartburn. They concluded that the test is not ready for clinical application due to frequently positive Peptest in healthy controls. Seventy-two percent had at least one sample with pepsin concentrations > 210 ng/mL (168). These results are in line with our study. Fifty-two % of our healthy controls had at least one salivary sample with pepsin concentration > 210 ng/mL.

8.6 General discussions

One of the main challenges when evaluating the role of GER in the pathogenesis of CRS are the sensitivity and specificity of the diagnostic procedures of the former. To comply with this, we have tried to combine different diagnostic strategies.

By doing so, we have obtained new insight in the field. However, evidence for a causality between GER and CRS is still lacking. CRS is a disease of multifactorial origin. Hence, a confirmation of a single etiological factor, which may apply to a limited number of these patients, may be difficult. We believe that future progress in this field depends on improved diagnostic procedures for EER, and further investigations on the effect of current and coming therapeutic strategies for GER in CRS.

9. Conclusion

The high SNOT- scores in the patients with GERD compared to healthy controls indicate a high incidence of CRS in GERD and a possible link between the two. This is supported by the findings of higher incidence of abnormal gastro esophageal reflux in CRS-patients compared to asymptomatic controls, in particular the abnormal proximal extent of the refluxate in the patients. The pathological mechanism may be either a vasomotor reflex from the proximal esophagus to the upper airway or a direct toxic effect of the refluxate on the airway mucosa. Peptest could not confirm the latter alternative, although it could not exclude its existence.

High concentrations of pepsin in nasal secretion and saliva in both CRS-patients and controls, and the lack of correlation between proximal reflux and positive Peptest indicated a limited validity of the test for this purpose.

As CRS is a disease of a multifactorial etiology, the evaluation of GER in CRS is difficult, but important.

10. Future perspectives

There is still a debate to what extent and how GER could be a contributing factor for CRS. We need more research in this area, and there are several unmet research questions for scientists to address.

The twenty-four-hour impedance pH-monitoring showed significantly more reflux in patients having CRS, compared to healthy controls. We hoped to see that the Peptest, as a quick and easy-handled diagnostic procedure would confirm this. Then, oto-rhino-laryngologists would have had a simple tool for selecting the patients with refractory CRS and GER to optimize the treatment. Unfortunately, Peptest does not appear to have the qualities necessary to be that tool. Hence, the search for alternatives should continue.

The novel diagnostic procedure, esophageal mucosal impedance (MI), measuring epithelial damage caused by reflux, may contribute with valuable information in future research. The MI, in combination with improved quantitative pepsin measuring and twenty-four-hour impedance pH-monitoring, may also provide more insight in the role of GERD in CRS.

Reflux may depend on lifestyle. As e.g. obesity and some foods may increase it, the way we are living our lives has an impact on GER. Lifestyle intervention may increase the pressure of LES and reduce the frequency of TLESRs. Weight loss is well documented to improve GERD, but data on the efficacy of elimination of certain predisposing foods is lacking. Further, to what extent lifestyle interventions recommended in the treatment of GERD may favorably affect GERD-related CRS is unknown. This should be explored.

There are cases of refractory CRS where reflux may be involved, and where medical measures against the sino-nasal disorder as such nor against GER solve the problem. Anti-reflux surgery is performed on selected patients suffering from GERD aiming to

improve the efficacy of LES. To what extent patients with reflux-induced CRS may benefit from such procedures remains unknown. It should be investigated.

The pathophysiological link between GER, even proximal GER, and the local effects in the sino-nasal mucosa, has not been studied. Studies on animal models, healthy subjects or patients looking at the effects of esophageal perfusion of acid, non-acid gastric solutions or water on nasal mucosal blood flow, may give insight into the role of vasomotor reflexes. This is technically possible by using laser Doppler flowmetry.

Based on the result in this thesis, it is reasonable to assume that reflux is involved in the pathogenesis of CRS in certain individuals. Awareness of this among doctors treating CRS-patients not responding to conventional treatment seems limited. Thus, some patients may be excluded from optimal treatment. This leaves a responsibility with the scientists behind these investigations to share this information in medical fora nationally and internationally.

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12. Errata

Errata for
The role of Gastroesophageal Reflux in Chronic Rhinosinusitis

Elin-Johanne Katle

Thesis for the degree philosophiae doctor (PhD)

at the University of Bergen

27/5-19 Elin-Johanne Katle
(date and sign. of candidate)

27.05.2019
(date and sign. of faculty)

Errata

Page 25 Missing reference number: "*Bousquet, Bachert et al. 2009" corrected to "26"*

Page 25 Missing reference number: "*Prokopakis, Vlastos et al. 2014" corrected to "28"*

13. Supplementary/ Questionnaires

13.1 Instructions for 24-h impedance pH-monitoring and pepsin measurement

24 timers pH- impedansmåling og Pepsinmåling

Navnelapp:

Undersøkelsesdato: _____ Start kl: _____ Stopp kl: _____

Plassering: _____

Liggende stilling:

1. Fra kl. _____ til kl. _____
2. Fra kl. _____ til kl. _____
3. Fra kl. _____ til kl. _____

Måltider:			Hva spiste du?
1. Påbegynt kl. _____	Avsluttet kl. _____		_____
2. Påbegynt kl. _____	Avsluttet kl. _____		_____
3. Påbegynt kl. _____	Avsluttet kl. _____		_____
4. Påbegynt kl. _____	Avsluttet kl. _____		_____
5. Påbegynt kl. _____	Avsluttet kl. _____		_____
6. Påbegynt kl. _____	Avsluttet kl. _____		_____
7. Påbegynt kl. _____	Avsluttet kl. _____		_____
8. Påbegynt kl. _____	Avsluttet kl. _____		_____
9. Påbegynt kl. _____	Avsluttet kl. _____		_____
10. Påbegynt kl. _____	Avsluttet kl. _____		_____

Symptomer/ kommentarer:

Registreringsknapper:

- Knapp 1: Oppstot
- Knapp 2: Brystbrann
- Knapp 3: Hoste
- Notater: Kremting/ behov for å rense halsen

Noter også (med evt klokkeslett): raping og klumpfornemmelse i svelg
Andre notater:

Pepsinmåling:

Du har fått utlevert 6 glass .
Vi ønsker ca 1 ml sekret i hvert glass. Dersom det er vanskelig å lage 1 ml nesesekret, så kan du «dra» 0,5 ml sterilt vann inn i hvert nesebor (trekk godt inn) før du snyter deg. Vær snill å noter eventuell bruk av sterilt vann. Noter klokkeslett for prøvetaking.

A1: Nesesekret ca 1 time etter stort måltid. Sterilt vann i nesen?: ja/ nei (sett ring rundt riktig)
kl: ……
B1: Oppspytt ca 1 time etter stort måltid
kl: ……
A2: Nesesekret ca 1 time etter stort måltid. Sterilt vann i nesen?: ja/ nei (sett ring rundt riktig)
kl.: ……
B2: Oppspytt ca 1 time etter stort måltid
kl: ……
A3: Nesesekret rett etter at du har stått opp om morgenen (innen 15 minutt) Sterilt vann i nesen?: ja/ nei (sett ring rundt riktig)
kl: ……
B3: Oppspytt rett etter at du har stått opp om morgenen (innen 15 minutt)
kl: ……

Vennligst ikke bruk nesespray disse 24 timene som registreringen pågår.

Rist glasset godt etter at sekret er plassert i glasset. Glasset bør oppbevares i kjøleskap frem til du leverer det inn når vi tar ut sonden etter 24 timer.

13.2 Twenty-item Sino-Nasal Outcome Test (SNOT-20)

Utfylling av vedlagt skjema for Sino-nasal-outcome-test

A:
Til venstre i spørreskjemaet finner du en rekke med 20 symptomer som er vanlige i forbindelse med kroniske nese-bihule-plager. Til høyre for disse symptomene finner du ruter med tall fra 0 til 5. Disse tallene angir alvorlighet av symptomene:

0 = Ingen plager
1 = Meget milde plager
2 = Lette plager
3 = Moderate plager
4 = Kraftige plager
5 = Verst tenkelige plager.

Det vi ber deg gjøre er å ta for deg hvert enkelt symptom, og sette en ring rundt det tallet som angir grad av det enkelte symptom slik du opplever det.

For eksempel:
Har du ingen problemer med nysing setter du en sirkel i rubrikken med 0 på linjen med spørsmål 2. Har du moderate problemer med at du er trist på grunn av problemene dine setter du en sirkel i ruten med 3-tallet på linjen med spørsmål 19.

B:
Helt til høyre finner du en kolonne med små ruter. Vi ber deg prøve å angi hvilke 5 av disse 20 problemene som plager deg mest ved å sette et kryss i ruten utenfor hvert av disse symptomene.

SINO-NASAL OUTCOME TEST

Nedenfor finner du en liste over symptomer og sosiale/følelsesmessige konsekvenser av din neselidelse. Vi vil gjerne vite mer om disse problemene, og vil være takknemlig hvis du vil besvare nedenstående spørsmål etter beste evne. Det er ikke noen riktige eller feile svar, og bare du kan gi oss den rette informasjonen. Vær vennlig å gradere dine problemer med utgangspunkt i situasjonen de siste to uker. Takk for at du vil delta.

A. Med utgangspunkt i hvor uttalt problemet er når det oppstår og hvor ofte det opptrer, bes du angi hvor "ille" det er ved å markere med sirkel det tallet som best svarer til det du føler, ut fra denne skala →→→→→→→	0: Ingen problemer	1: Meget milde problemer	2: Milde eller lette problemer	3: Moderate problemer	4: Kraftige problemer	5: Problemene er så kraftige som det er mulig	**B.** Viktigste punkter (maks 5 punkt)
1. behov for å pusse nese	0	1	2	3	4	5	
2. nysing	0	1	2	3	4	5	
3. rennende nese	0	1	2	3	4	5	
4. hoste	0	1	2	3	4	5	
5. renning bak i svelget	0	1	2	3	4	5	
6. tykt sekret fra nesen	0	1	2	3	4	5	
7. tetthet i ørene	0	1	2	3	4	5	
8. svimmelhet	0	1	2	3	4	5	
9. øresmerter	0	1	2	3	4	5	
10. smerter/trykk i ansiktet	0	1	2	3	4	5	
11. vanskelig å falle i søvn	0	1	2	3	4	5	
12. våkner om natten	0	1	2	3	4	5	
13. mangel av god nattesøvn	0	1	2	3	4	5	
14. trøtt når du våkner	0	1	2	3	4	5	
15. kraftesløshet	0	1	2	3	4	5	
16. nedsatt produktivitet	0	1	2	3	4	5	
17. nedsatt konsentrasjon	0	1	2	3	4	5	
18. frustrert/rastløs/irritabel	0	1	2	3	4	5	
19. trist	0	1	2	3	4	5	
20. flau	0	1	2	3	4	5	

B.
Vær vennlig å markere de viktigste punktene som påvirker din helsetilstand (maksimum 5 punkter)

13.3 Gastroesophageal Reflux Disease Questionnaire (GerdQ)

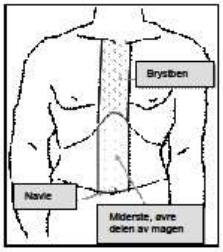
GerdQ

Spørreskjema for pasienter med øvre gastrointestinale symptomer

Svar på spørsmålene ved å krysse av i **én** rute per rad.

Når du tenker over symptomene du har hatt de siste 7 dagene, hvor ofte opplevde du følgende

	Aldri	1 dag	2-3 dager	4-7 dager
1. Hvor ofte opplevde du en brennende følelse bak brystbenet (halsbrann)?	☐	☐	☐	☐
2. Hvor ofte opplevde du at mageinnholdet (væske eller mat) kom opp i halsen eller munnen (oppstøt)?	☐	☐	☐	☐
3. Hvor ofte opplevde du smerte i midterste, øvre del av magen?	☐	☐	☐	☐
4. Hvor ofte var du kvalm?	☐	☐	☐	☐
5. Hvor ofte opplevde du at det var vanskelig å få en god natts søvn på grunn av halsbrann og/eller oppstøt?	☐	☐	☐	☐
6. Hvor ofte tok du annen medisin for halsbrann og/eller oppstøt i tillegg til det som legen har anbefalt? (f.eks. Titralac, Link, Novalucid)	☐	☐	☐	☐



GerdQ – Norway-Norwegian.
Version 1.

29 May 2008

13.4 Visual Analogue Scale (VAS-CRS)

Navn: Diagnose:

Alder:

SSK-skjema for nese-bihule-symptomer

Høyde: Vekt: BMI: Allergi: Astma: Yrke:

Antall sigaretter om dagen: I hvor mange år:

Tett nese	Helt åpen	Helt tett
Munnpusting	Aldri	Alltid
Snorking	Aldri	Alltid
Pustepauser under søvn	Aldri	Alltid
Renning fra nesen	Aldri	Alltid
Hodepine	Aldri	Alltid
Smerter i tenner/midtansikt	Aldri	Alltid
Bihulebetennelse	Aldri	Alltid
Hoste	Aldri	Alltid
Nysing	Aldri	Alltid
Nedsatt allmenntilstand	Aldri	Alltid
Nedsatt luktesans	Aldri	Alltid

14. Original publications

III

Rhinology Online

ORIGINAL CONTRIBUTION

OPEN ACCESS

Nasal and salivary pepsin as a biomarker for gastro-esophageal reflux in chronic rhinosinusitis*

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Abstract

Background: Gastro-esophageal reflux (GER) may be a contributing factor for some patients with chronic rhinosinusitis (CRS). The aim of the present study was to investigate if Peptest, an immunoassay for pepsin detection, could be used as a biomarker for GER in CRS.

Methodology: Peptest was used to analyse 3 saliva and 3 nasal samples for pepsin A in 62 CRS-patients and 62 age and gender matched healthy controls. The results were correlated to 24-hour impedance pH-monitoring and symptom questionnaires.

Results: Patients with CRS did not have more abnormal Peptest measures compared to healthy controls, 39 patients and 48 controls, respectively. The presence of abnormal Peptests did not correlate to proximal reflux in CRS-patients. Patients with high GerdQ scores did not have more positive Peptests than those without.

Conclusions: These results question the value of Peptest as screening tool for GER in CRS.

Key words: Chronic rhinosinusitis, gastro-esophageal reflux, pepsin, Peptest

Introduction

Chronic rhinosinusitis (CRS) is a common chronic condition affecting up to 15 % of the adult population [(1)]. It influences quality of life and working capacity by the inflammation of the mucosa of the nose and paranasal sinuses. The aetiology of the inflammatory process is not completely understood, but a common hypothesis is that inappropriate immune responses to foreign agents results in CRS. Stimulation of T cells by *Staphylococcus aureus* protected in biofilms is an example of such immune response. Whether severe cases of non-allergic rhinitis, allergic rhinitis, occupational rhinitis and CRS not responding to conventional treatment, could share a common underlying pathophysiology, is unclear [(2)].

The lack of etiological clues impairs the development of evidence based therapeutic strategies. The high number of patients with CRS failing to respond to medical and surgical treatment represents a major challenge both for the individual patients as such, but also for general practitioners as well as for specialists, and for society in general.

The role of Gastro-esophageal Reflux (GER) in upper airway disorders including CRS has been debated for decades [(3-5)]. GER has an established role in the pathogenesis of dental erosions [(6)], asthma [(7)], chronic cough [(8)], laryngitis [(9)] and possibly also in chronic otitis media [(10)]. Its role in CRS is unclear [(3, 11, 12)]. In the European Position Paper on Rhinosinusitis and Nasal Polyps 2012 (EPOS 2012) it is therefore stated that more research is needed to enlighten the role of GER in CRS [(13)].

In a recent study, we demonstrated that patients with gastro

esophageal reflux disease (GERD) had high scores on SNOT-20, a questionnaire for sino-nasal quality of life [14]. In another study, we found that abnormal gastroesophageal reflux was significantly more prevalent in CRS-patients than in healthy subjects as evaluated by 24 -hour esophageal impedance pH monitoring, the gold standard for the investigation of GER [15].

Twenty-four-hour impedance pH-monitoring is a resource-demanding procedure, and not easily available. Alternative diagnostic methods are pH- monitoring only or endoscopic examinations. The former will not detect episodes of non-acid reflux, and the latter will only detect those with visible pathology in the esophagus. Thus, it is warranted to find alternative and still valid methods for investigation of GER in CRS.

Reflux of gastric contents through the esophagus into the airways includes acid, pepsin, mucus, bile acids, pancreatic enzymes, and remnants of food and drinks. Pepsin has been used as a diagnostic biomarker of GERD. A study of paediatric patients demonstrated an association between pepsin in saliva and findings at 24- hour impedance pH- monitoring [16]. Also, pepsin concentrations in nasal lavage fluid were high, and correlated well with esophageal acid exposure [17]. However, these results were not reproduced in another study which concluded that detection of salivary pepsin was an imprecise method at least for investigation of reflux in children with CRS [18].

The aim of the present study was to evaluate if Peptest, a widely used immunoassay method for pepsin detection, could be used as a biomarker of GER in CRS. Furthermore, we wanted to evaluate if pepsin could be measured in nasal secretions and if so, compare the results with measures from healthy controls.

Methodology

Patients and healthy controls

This was a prospective controlled age and gender matched study, performed on an outpatient basis. Consecutive patients above 18 years of age, referred to the ENT department, Stavanger University Hospital and diagnosed with CRS with or without polyps according to the EPOS 2012 criteria [13] were invited to participate. Sixty two CRS patients were included, 33 men and 29 women. A similar number of age and gender matched healthy controls were recruited at different jobs, among patient's friends, neighbours, colleagues and among the investigator`s friends, family, colleagues and neighbours (Table 1). Median age in patients and healthy controls was 46 and 46.5 respectively (p=0.81). The same investigator handled all patients and healthy controls.

Exclusion criteria were use of anti-reflux medication on a daily basis. Symptoms of reflux were not an exclusion criteria per se. Nasal corticosteroids were not allowed the day of investigation.

Table 1. Subject characteristics.

	CRS-patients	Healthy controls
Age, years	46	46.5
Gender, men/ women	33/ 29	33/ 29
Smoking	10	1
BMI, median	25.1	25.9
Self-reported asthma	18	2
Ongoing treatment of reflux	0	0
Self-reported allergy	16	8

CRS= Chronic rhinosinusitis, BMI= Body Mass Index

Peptest

Peptest® (RD Biomed Ltd, Cottingham, UK) is an enzyme linked immunoassay for detection of pepsin in saliva. This non-invasive test is based on a lateral flow device technology. It consists of antibodies to human pepsin and is specific to pepsin A, the isoform secreted only in the stomach [19, 20]. A control band on the device indicates a correctly performed test and a second line indicates presence of pepsin, in the sample, defining a positive or negative test. The intensity of this line is proportional to the quantity of pepsin, and by using a reader, LFDR101, the concentration of pepsin in ng/mL is obtained [20]. Both the sensitivity and specificity of Peptest has been claimed to be 87 % [21], but recent meta-analysis suggests the sensitivity and the specificity to be lower, 64% and 65% respectively [22]. The lower limit for detection of pepsin with Peptest is 16ng/ mL given by the manufacturer [20]. To the best of our knowledge, Peptest has not previously been used on nasal secretions.

CRS patients and healthy controls delivered 3 tubes with saliva and 3 tubes with nasal secretion (roughly 1 mL in each) for pepsin analysis. We strictly followed the recommendations from the manufacturers; "Sample one should be taken in an upright position within 15 minutes of waking up from overnight sleep before eating or brushing teeth, the second sample should be taken one hour following the main meal of the day and the third sample should be taken one hour following the next main meal of the day" [23]. Subjects without rhinorrhoea used 0,5 mL sterile water for irrigation of each nostril to be able to deliver samples. The tubes for investigation contained 0,5 mL of 0,01 M citric acid for conservation, and the participants stored the samples in a refrigerator until analysis within 7 days, as recommended by the manufacturer [21]. Each sample was centrifuged at 5000 rpm for 5 minutes, 80 µl of the supernatant was extracted, and 240 µL migration buffer was added. This was vortex-mixed for 10 seconds before 80 µL was transferred to the pit of the Peptest device. The test was read as positive or negative after 15 minutes and quantification done by using the LFDR reader.

The same investigator performed all the tests on both CRS patients and healthy control subjects.

Table 2. Symptom scores in patients with chronic rhinosinusitis and healthy controls.

	CRS-patients	Healthy controls	p*
GerdQ, median (IQR)	6 (5.3-7.0)	6 (5.5-6.0)	0.25*
Number participants with GerdQ ≥ 8	12	3	0.04**
VAS-CRS, median (IQR)	50.5 (31.7- 65.8)	15.5 (8.8- 20.1)	< 0.001*
SNOT-20, median (IQR)	34.5 (20.3- 52.5)	4 (2.0- 10.0)	< 0.001*

Questionnaires were available for 60 matched pairs of patients and controls. *Wilcoxon test for paired samples, **McNemar test for paired samples, CRS= Chronic rhinosinusitis, IQR= Interquartile Range, GerdQ= Gastroesophageal Reflux Disease Questionnaire, VAS-CRS = Visual Analogue Scale for chronic rhinosinusitis, SNOT-20= 20-item Sinonasal Outcome Test.

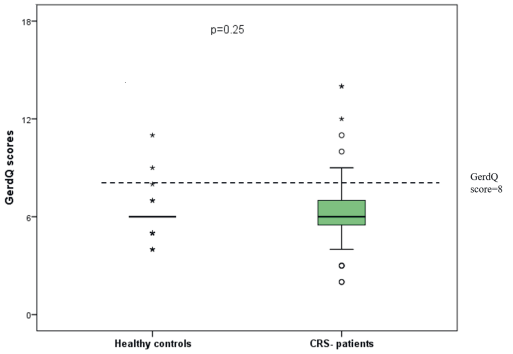

Figure 1. GerdQ scores in healthy controls and CRS-patients. No significant difference in GerdQ, Gastroesophageal Reflux Disease Questionnaire, scores between CRS-patients and healthy controls, Wilcoxon, p=0.25. GerdQ is a validated six item, self- administered questionnaire, scoring symptoms of reflux and dyspepsia separately, with reflux symptoms increasing the score and dyspepsia decreasing it. Absence of any such symptoms gives a total score of six and scores above eight indicate reflux disease. Three healthy controls and 12 patients had a GerdQ score ≥ 8, which is the level indicating GERD (p=0.08). CRS= Chronic rhinosinusitis.

24-hour combined pH-impedance monitoring

Forty-six patients completed 24-h combined pH-impedance monitoring, with recordings for 18 - 26 hours. The impedance pH-catheter (ZAN-BG-44, Sandhill Scientific, Inc; Highland Ranch, CO, USA) was positioned trans-nasally into the oesophagus, with the proximal pH-electrode located 5 cm above the lower esophageal sphincter and the impedance electrodes 2, 4, 6, 8, 10, 14, 16 and 18 cm above the lower esophageal sphincter [15].

Questionnaires

Validated questionnaires were used to evaluate the clinical symptoms of GERD and CRS in patients and controls:
Visual Analogue Scale (VAS-CRS) is a psychometric response scale used to measure subjective sino-nasal symptoms [13]. The 20-item Sinonasal Outcome Test (SNOT-20) is a validated self-administered quality of life instrument specific for patients with symptoms of rhinosinusitis [24]. The Gastro-Esophageal Reflux Disease Questionnaire (GerdQ) was used to record classical symptoms of GERD. It is a validated six item, self- administered questionnaire, scoring symptoms of reflux and dyspepsia separately, with reflux symptoms increasing the score and dyspepsia decreasing it. Absence of any such symptoms gives a total score of 6 and scores above 8 indicate reflux disease [25].
The Peptest scores were compared to GerdQ and VAS-CRS and SNOT-20. GerdQ, VAS-CRS and SNOT-20 in CRS-patients and healthy controls were also compared.

Statistical analysis

As data were not normally distributed, non-parametric statistics was used. Summary statistics are reported as median and interquartile range (IQR) for continuous variables and numbers and percentages for categorical variables. Differences between patients and controls were examined using the paired samples Wilcoxon test for continuous data and McNemar test for categorical data. Correlations between variables where calculated using Spearman`s Rank Correlation (rho).
The statistical analyses were done using SPSS ver. 24.0 (Statistical Package for Social Science, Chicago, IL, USA).

Ethics and approval

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by Regional Norwegian Committee for Medical and Health Research Ethics approved the study (REK Vest 2010/2030). All participants were included after written informed consent.

Results

Sixty-two CRS patients were included, 33 men and 29 women, and 62 controls (Table 1). There was no significant difference in Body Mass Index (BMI) between the CRS-patients and healthy subjects, median 25.1 and 25.9 respectively (p=0.46) (Table 1). Questionnaires were available for 60 matched pairs of patients and controls. Total GerdQ scores were not significantly higher in the CRS-patients compared to the healthy controls (p=0.25) but 12 patients had GerdQ score ≥ 8, which is the level indicating GERD, compared to 3 in the control group (p=0.04) (Table 2). The patients had significantly higher SNOT-20 and VAS-CRS scores, compared to the controls (Table 2).
There was no significant difference in number of positive

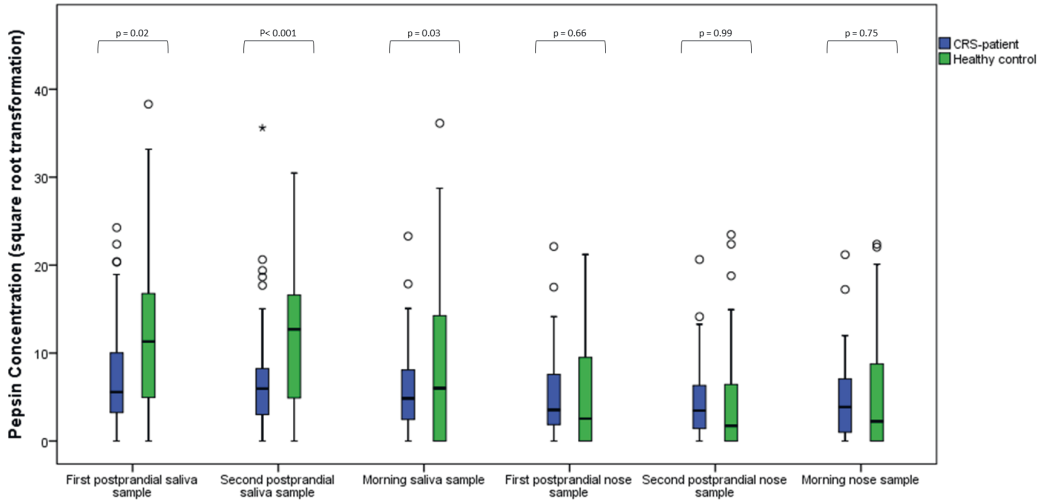

Figure 2. Pepsin concentration in nasal secretion and saliva in healthy controls and CRS-patients. Pepsin level in nasal secretion and saliva measured one hour after two different meals and within fifteen minutes after waking up in the morning. There were no significant differences in pepsin concentration in nasal samples between CRS-patients and healthy controls, but significantly higher pepsin concentration in saliva in healthy control subjects compared to CRS-patients, paired sample Wilcoxon test. The concentrations are square root transformed for improved readability.

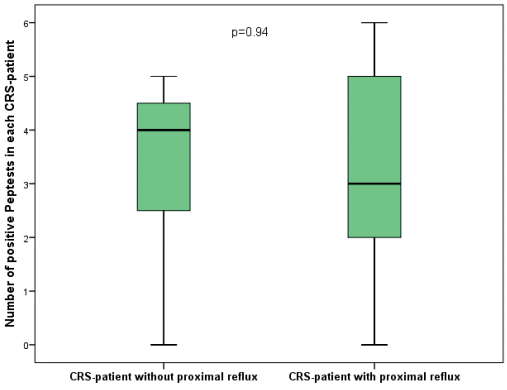

Figure 3. Total number of Peptest positive samples per CRS-patient with and without proximal reflux, diagnosed with 24-hour pH-impedance monitoring. There was no significant difference, p= 0.94.

salivary Peptests in patients and healthy controls (Table 3). Abnormal Peptest results in saliva samples, when defined as two or more positive Peptests or Peptest levels in one test ≥ 100 ng was found in 39 patients and 48 controls (p= 0.08). The concentration of pepsin was significantly higher in the saliva of healthy controls compared to CRS-patients (Table 3, Figure 2). There were large variations in pepsin levels both in patients and controls, particularly in the latter.

There was significantly higher number of positive Peptests in the second postprandial nasal sample in patients compared to healthy controls (p=0.02), but not in the other samples (Table 3), and the levels of pepsin in nasal samples did not differ between the two groups (Table 3, Figure 2).

There was no significant difference in number of positive Peptests between patients with abnormal proximal reflux compared to those without (p=0.94) (Figure 3), and no correlation between the number of positive Peptests and number of proximal reflux (Spearman rho 0.13, p=0.42), nor between the number of positive Peptests and number of distal acid reflux episodes (Spearman rho 0.06, p=0.71). CRS patients with high GerdQ scores did not show higher concentration of pepsin in saliva or nasal secretion and number of positive tests was not significantly different between the two groups (p= 0.71). There was no correlation between GerdQ scores and number of positive Peptest in the healthy controls (Spearman rho 0.17, p=0.18) or patients (Spearman rho 0.15, p=0.24).

Discussion

In this study we have demonstrated that patients with CRS did not have more pepsin A in saliva or nasal secretions as measured by Peptest compared to healthy controls. CRS patients with abnormal proximal reflux did not have more samples positive for Peptest than patients without. As a group, there were more patients with high GerdQ scores for classical GERD symptoms compared to the healthy controls. However, less than 40% of patients with EER may have the classical symptoms of reflux,

Table 3. Pepsin concentration in saliva and nasal secretions, as measured with Peptest.

	Number of positive Peptests (%)			Pepsin concentration, ng/ mL, median (IQR)		
	CRS-patients	Healthy controls	P*	CRS-patients	Healthy controls	P**
First postprandial saliva sample	35 (67.3)	34 (65.4)	1.00	31.0 (10.0-101.0)	128.0 (24.3-282.0)	0.02
Second postprandial saliva sample	34 (60.7)	37 (66.1)	0.69	35.5 (8.5-68.3)	161.5 (21.5-278.5)	<0.001
Morning saliva sample	30 (51.7)	27 (46.6)	0.71	23.5 (6,0-68.3)	36.0 (0.0- 238.5)	0.03
First postprandial nose sample	27 (47.4)	17 (29.8%)	0.11	12.5 (3.3-58.8)	6.5 (0.0-93.3)	0.66
Second postprandial nose sample	26 (45.6)	13 (22.8)	0.02	12.0 (1.0- 42.1)	3.0 (0.0- 44.0)	0.99
Morning nose sample	26 (42.6)	17 (27.9)	0.15	15.0 (1,0-52.5)	5,0 (0,0-80.0)	0.75

Peptest results in saliva and nose samples collected at three different times during a day in CRS patients and healthy controls. There were significantly more positive Peptests in CRS-patient`s second nasal postprandial sample but no significant difference between the other groups regarding number of positive tests. There was significantly higher pepsin concentration in saliva samples from healthy controls, but no significant difference in pepsin concentrations in nasal samples between CRS-patients and healthy controls regarding pepsin concentration. The number of paired samples available varies from 48 to 61. CRS= Chronic rhinosinusitis, IQR= Interquartile range, *Categorical data tested with McNemar test, ** Continuous data tested with Wilcoxon test.

meaning that the questionnaire does not have an important role in the evaluation of these conditions. Patients with high GerdQ scores or verified proximal reflux did not have more positive Peptests than the controls. These findings question the validity of the Peptest in the evaluation of GER in CRS.

Pepsin is a proteolytic enzyme responsible for digestion of proteins in the stomach. As pepsin A originates from pepsinogen secreted from gastric chief cells in the stomach, the finding of pepsin A in the esophagus, mouth or respiratory tract should be a diagnostic marker of reflux [26]. The nasal mucosa has limited protective capacity against the refluxate, and could be more sensitive to its injurious influence [12]. Pepsin is most harmful in its acidic state. However, it can cause injury with a pH up to 6,5 and is not irreversibly denatured until pH reaches 8. This implies that when pH in refluxate is above 4, and thus not detected by pH monitoring, there can still be active pepsin in the refluxate. Besides, theoretically, inactive pepsin can be reactivated and become harmful by a decrease in pH due to acid drinking or later acidic reflux events [27].

The high number of positive Peptests in both patients and healthy controls in this study supports the concept of physiological, mainly postprandial reflux. Every healthy individual refluxes small amounts of gastric contents, mainly after meals, most of them without having symptoms, and without having GERD [11]. Less is known about reflux to the airways in healthy subjects. The high number of positive Peptests both in those with and without CRS may indicate individual differences in sensitivity to refluxate including pepsin, i.e. a certain level of pepsin may create disease in some individuals, but not in others. This theory has been supported by pH- impedance monitoring of the esophagus combined with symptom registration, with an estimation of Symptom Association Probability (SAP). SAP gives an indication of a time correlation between symptoms and reflux episodes, and it has been shown that exposure to refluxate that normally is considered physiological, may give symptoms in some patients [28]. Thus, it may be speculated whether the same differences in vulnerability also exists in the nose, whether a certain level of pepsin may contribute to the development of CRS in certain predisposed individuals, but not in others. The lower concentration of pepsin in patients compared to controls may partly be explained by gastroesophageal reflux inducing hyper salivation [29]. The phenomenon has also been described in healthy persons when stimulating the esophagus with hydrochloric acid [30]. Hyper-salivation may dilute the concentration of pepsin in patients and thus act as a confounder in this study.

Peptest is supposed to give snapshot-information about the presence or absence of pepsin in saliva and nasal secretions. The concentration of pepsin in saliva varies during the day and decreases rapidly after an episode of reflux [31]. Thus, saliva samples should be obtained soon after reflux events to detect the pepsin [16]. Inappropriate timing can reduce real-life sensitivity of the Peptest though it has high technical sensitivity [32]. Reflux appears mostly after meals, and corresponding symptoms of GERD is significantly highest 1 to 2 hours post-prandially [31]. In the present study, we tested for pepsin 3 times a day, determined by time of waking up and time of meals and not by symptomatic reflux episodes. Hence, there could be episodes of reflux not detected with Peptest both in CRS-patients and controls, most likely more frequently in the former. That this is a valid assumption is supported by the fact that pH-impedance of the esopha-

gus, monitoring gastro-esophageal reflux continuously over a prolonged period of time, demonstrated both an increased incidence and severity of reflux in CRS-patients [15]. Then it may be discussed to what extent detection of refluxate of gastric content, including pepsin, in the esophagus implies its presence and pato-physiologic role even in the upper airways. For the time being, there is no conclusive answer to that question. Two different pathophysiological mechanisms have been proposed to explain how GERD may contribute to airway manifestations, through a vagal reflex or a direct toxic effect. As to CRS, the theory is that a neuronally induced nasal mucosal oedema gives ostial obstruction [33]. Our results, with no important difference in pepsin in nasal secretions and saliva between CRS-patients and controls and no significant difference in pepsin in nasal secretion or saliva samples between CRS patients with proximal reflux or not, may be in line with this, though reservations have to be made due to the limited specificity of the Peptest.

The present study raises important questions about the validity and utility of Peptest as a screening method for extra-esophageal reflux as also discussed by others [22]. One can also question its usefulness in diagnosing GERD in general, since patients with abnormal gastroesophageal reflux had no more positive tests than those without. We strictly followed the recommendations from the manufacturers and found high levels of pepsin in both CRS patients and healthy controls, with even significantly higher levels of pepsin in saliva in controls compared to patients. Accordingly, it appears unlikely that methodological flaws should affect the present results and explain the high levels of pepsin in the control group. As data being the basis for calculation of the specificity of the test varies, and tends to be lower than previously assumed [22], it is relevant to question the practical specificity of the test when used as a diagnostic tool for GERD in upper airway diseases. If Peptest had been a reliable method to diagnose extra esophageal manifestation of GERD, we would also expect more positive tests in patients having proximal reflux as evaluated by 24-h impedance pH monitoring compared to those without. The fact that CRS-patients, even those with severe GER, did not have more pepsin in saliva and nasal secretion than control subjects, supports the speculations about the utility of Peptest in clinical practice. However, we cannot rule out that it may have a role in cases with classical symptoms of reflux, and where test samples can be collected immediately after a reflux episode.

Conclusion

As measured by Peptest, we did not find more pepsin in saliva or nasal secretions in CRS- patients than in healthy controls, nor in those with those with high GerdQ scores or verified proximal reflux, indicating a limited validity of the Peptest as screening tool for GER in CRS.

Abbreviations

CRS= Chronic Rhino Sinusitis, GERD= Gastro Esophageal Reflux Disease, GER= Gastro Esophageal Reflux, VAS= Visual Analog Scale, SNOT-20= Sino Nasal Outcome Test-20, GerdQ= Gastro Esophageal Reflux Disease Questionnaire, BMI= Body Mass Index.

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Authorship contribution

EJK, JGH, RO and SKS designed the study. EJK collected the data. EJK analysed and interpreted the data together with JGH, RO, JTK and SKS. EJK wrote the manuscript with assistance from all the authors who also accepted the final version.

Conflict of interest

There is no conflict of interest to report.

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