Acid Reflux at the Gastroesophageal Junction. Pathophysiological and Diagnostical Aspects

Hall, Mats

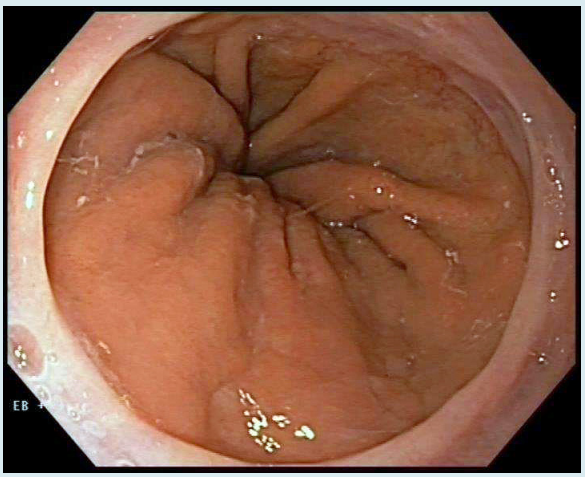
2018

Document Version:
Publisher's PDF, also known as Version of record

Acid Reflux at the Gastroesophageal Junction

Pathophysiological and Diagnostical Aspects

MATS HALL | FACULTY OF MEDICINE | LUND UNIVERSITY



Printed by Media-Tryck, Lund 2018 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Surgery, SUS
Clinical Sciences Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2018:90
ISBN 978-91-7619-656-4
ISSN 1652-8220

ACID REFLUX AT THE GASTROESOPHAGEAL JUNCTION

PATHOPHYSIOLOGICAL AND DIAGNOSTICAL ASPECTS

Mats Hall

LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty Medicine, Lund University, Sweden.
To be defended at föreläsningssal F2,

Centralblocket, Skånes Universitetssjukhus, Lund .

Date 2018-05-30 and time 13.00.

Faculty opponent

Docent Björn Lindkvist

Sahlgrenska Akademin
Göteborgs Universitet

Organization LUND UNIVERSITY Department of Surgery, SUS Clinical Sciences Lund	Document name DOCTORAL DISSERTATION Date of Issue 180530
Author(s) Mats Hall, MD	Sponsoring organization

Title and subtitle

Acid reflux at the gastroesophageal junction- Pathophysiological and diagnostical aspects

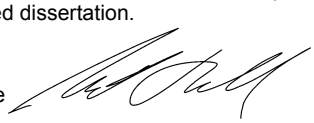
Abstract

Gastroesophageal reflux disease (GERD) has a prevalence of 20% based on weekly symptoms of heartburn and regurgitation. As reflux symptoms are unspecific for GERD, an objective evaluation of the presence of the disease is often needed. Ambulatory pH monitoring is the most commonly used diagnostic method but it is limited by relatively poor sensitivity, especially in patients without esophagitis. The diagnostic accuracy of esophageal pH monitoring performed immediately above the squamocolumnar junction (SCJ) was compared with that of the conventional level in patients with symptoms of GERD and healthy volunteers. The degree of acid exposure at the SCJ is significantly underestimated by pH monitoring at the conventional level. Using a predefined specificity of 90%, the sensitivity of the pH test was significantly greater for pH monitoring performed immediately above the SCJ compared with that of pH monitoring at the traditional level. In patients without mucosal injury, the sensitivity improved significantly from 45% to 66% and was further improved with the combination of a positive SAP for the symptom of heartburn to 94%. The highly acidic “acid pocket” in the proximal stomach after a meal, suggested to be important in the pathophysiology of GERD was studied with a pH electrode positioned immediately above the SCJ. Distal postprandial reflux was characterized by short, rapidly cleared reflux events of a relatively low acidity and the lack of prolonged periods of postprandial acid exposure questions the hypothesis that the acid pocket extends into the most distal esophagus and its importance in the pathogenesis of GERD. As complications of GERD, such as esophagitis, metaplasia and adenocarcinoma most commonly occur in the area of the gastroesophageal junction (GEJ), the acid environment in this area is of particular interest for the pathophysiology of GERD. The pattern and degree of acid exposure was measured immediately above the SCJ and associations with the endoscopic appearance of the SCJ and the presence of specialized intestinal metaplasia (IM) was evaluated. The histologic finding of IM at a normal appearing SCJ was significantly associated with abnormal acid exposure and frequent reflux episodes, but not with Helicobacter Pylori infection. With increasing degree of irregularity of the SCJ, the frequency and duration of reflux episodes, the degree of distal esophageal acid exposure and the prevalence of abnormal acid exposure increased progressively and significantly, suggesting that the shape of the normal SCJ is even and that also minimal irregularities are a consequence of acid reflux, likely due to formation of small areas of metaplastic columnar mucosa..

Key words: GERD, wireless, pH monitoring, diagnostic accuracy, Intestinal metaplasia, ZAP grade

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language English
ISSN and key title: 1652-8220 Lund University, Faculty of Medicine Doctoral Dissertation Series 2018:90		ISBN 978-91-7619-656-4
Recipient’s notes	Number of pages 112	Price
	Security classification	

Signature  Date 180427

ACID REFLUX AT THE GASTROESOPHAGEAL JUNCTION

PATHOPHYSIOLOGICAL AND DIAGNOSTICAL ASPECTS

Mats Hall

LUND
UNIVERSITY

Cover photo by Mats Hall

e-mail: mats.hall@med.lu.se

Supervisor: Associate Professor Stefan Öberg
Department of Surgery, Helsingborg Hospital
Clinical Sciences, Lund
Lund University, Faculty of Medicine Doctoral Dissertation Series 2018:90

ISBN 978-91-7619-656-4
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2018

MADE IN SWEDEN

To my family because family means everything

Table of Contents

Abstract

Gastroesophageal reflux disease (GERD) has a prevalence of 20% based on weekly symptoms of heartburn and regurgitation. As reflux symptoms are unspecific for GERD, an objective evaluation of the presence of the disease is often needed. Ambulatory pH monitoring is the most commonly used diagnostic method but it is limited by relatively poor sensitivity, especially in patients without esophagitis. The diagnostic accuracy of esophageal pH monitoring performed immediately above the squamocolumnar junction (SCJ) was compared with that of the conventional level in patients with symptoms of GERD and healthy volunteers. The degree of acid exposure at the SCJ is significantly underestimated by pH monitoring at the conventional level. Using a predefined specificity of 90%, the sensitivity of the pH test was significantly greater for pH monitoring performed immediately above the SCJ compared with that of pH monitoring at the traditional level. In patients without mucosal injury, the sensitivity improved significantly from 45% to 66% and was further improved with the combination of a positive SAP for the symptom of heartburn to 94%. The highly acidic "acid pocket" in the proximal stomach after a meal, suggested to be important in the pathophysiology of GERD was studied with a pH electrode positioned immediately above the SCJ. Distal postprandial reflux was characterized by short, rapidly cleared reflux events of a relatively low acidity and the lack of prolonged periods of postprandial acid exposure questions the hypothesis that the acid pocket extends into the most distal esophagus and its importance in the pathogenesis of GERD. As complications of GERD, such as esophagitis, metaplasia and adenocarcinoma most commonly occur in the area of the gastroesophageal junction (GEJ), the acid environment in this area is of particular interest for the pathophysiology of GERD. The pattern and degree of acid exposure was measured immediately above the SCJ and associations with the endoscopic appearance of the SCJ and the presence of specialized intestinal metaplasia (IM) was evaluated. The histologic finding of IM at a normal appearing SCJ was significantly associated with abnormal acid exposure and frequent reflux episodes, but not with *Helicobacter Pylori* infection. With increasing degree of irregularity of the SCJ, the frequency and duration of reflux episodes, the degree of distal esophageal acid exposure and the prevalence of abnormal acid exposure increased progressively and significantly, suggesting that the shape of the normal SCJ is even and that also minimal irregularities are a consequence of acid reflux, likely due to formation of small areas of metaplastic columnar mucosa.

List of publications

This thesis is built upon the following papers in the text referred according to their roman numerals (I-V)

I. Wenner J, Hall M, Johansson J, Johnsson F, Öberg S. Wireless pH recording immediately above the squamocolumnar junction improves the diagnostic performance of esophageal pH studies. Am J Gastroenterol 2008;103:2977–85.

II. Guerrero Garcia Hall M, Wenner J, Öberg S. The combination of pH monitoring in the most distal esophagus and symptom association analysis markedly improves the clinical value of esophageal pH tests. Scand J Gastroenterol. 2016;51(2):129-36.

III. Hall M, Wenner J, Öberg S. The postprandial acid pocket does not extend into the oesophagus: Evidence from pH studies performed immidiately above the squamocolumnar junction. Scand J Gastroenterol. 2014;49(1):15-22.

IV. Hall M, Scherman, Wenner J, Öberg S. Intestinal metaplasia at the gastroesophageal junction is associated with gastroesophageal reflux but not with Helicobacter Pylori infection. Submitted.

V. Hall MG, Wenner J, Öberg S. The normal squamocolumnar junction is circumferentially even and minimal irregularities are manifestations of gastroesophageal acid reflux. Scand J Gastroenterol 2017;52(3):270-275.

Permissions

Abbreviations

AUC	AREA UNDER THE CURVE
BE	BARRETTS ESOPHAGUS
BMI	BODY MASS INDEX
GEJ	GASTROESOPHAGEAL JUNCTION
GERS	GASTRO ESOPHAGEAL REFLUX SYMPTOMS
GERD	GASTRO ESOPHAGEAL REFLUX DISEASE
HP	HELICOBACTER PYLORI
LAC	LOS ANGELES CLASSIFICATION OF ESOPHAGITIS
LES	LOWER ESOPHAGEAL SPHINCTER
MII	MULTICHANNEL INTRALUMINAL IMPEDANCE
NERD	NON EROSIVE ESOPHAGEAL REFLUX DISEASE
NCCP	NON-CARDIAC CHEST PAIN
PPI	PROTON PUMP INHIBITOR
QOL	QUALITY OF LIFE
OTC	OVER THE COUNTER
ROC	RECEIVER OPERATING CHARACTERISTICS
SCJ	SQUAMOCOLUMNAR JUNCTION
SAP	SYMPTOM ASSOCIATION PROBABILITY
SI	SYMPTOM INDEX
SSI	SYMPTOM SENSITIVITY INDEX
TLESR	TRANSIENT LOWER ESOPHAGEAL SPHINCTER RELAXATIONS
TNM	TUMOUR NODES METASTASIS CLASSIFICATION

Introduction

Gastroesophageal reflux disease (GERD) is a chronic disease representing one of the most common disorders of the gastrointestinal tract where between 10-20% of the adult population experience typical symptoms of reflux on a regular basis (1, 2). Defining GERD is difficult because of it´s heterogeneous nature and consequently there has been made several attempts do define the disease. In the latest by the Montreal consensus group in 2006, GERD was defined a condition including both symptoms and complications of refluxed gastric contents (3). GERD with associated complications share similarities in symptomatology with other diseases, such as cardiac disease, esophageal motor disorders, asthma but GERD also exist in an asymptomatic entity only to become apparent in the event of a complication causing symptoms.

In 2002, GERD was ranked as the 4 th most expensive disease of the gastrointestinal tract and in 2012 ranked the most common diagnosis of the gastrointestinal tract in the USA(4, 5).

Besides from patient suffering and high socioeconomic costs, the high prevalence combined with risk of serious complications, such as esophagitis, hemorrhage, strictures, the development of premalignant metaplasia, Barrett´s esophagus (BE) and adenocarcinoma in the distal esophagus makes the disease clinically important. BE, is associated with adenocarcinoma of the esophagus and patients with BE has a 30 times increased risk of developing esophageal adenocarcinoma compared with the normal population (6, 7). Importantly, the incidence of adenocarcinoma of the esophagus has shown a rapid un-comparable increase the last 4 decades and unfortunately the prognosis for patients developing adenocarcinoma in the esophagus in spite of treatment is poor (8, 9). The strong association between reflux symptoms and adenocarcinoma of the esophagus is firmly established but the underlying pathological mechanisms are despite much research effort still largely unknown (10).

The main method to objectively evaluate the degree of gastroesophageal reflux is by measuring esophageal acid exposure during ambulatory pH monitoring. The initial and most common encounter with the GERD patients is in primary care, where ambulatory pH monitoring is practically unavailable. PH monitoring is also limited by methodological problems and a relatively low diagnostic accuracy, in particular in patients without esophagitis and patients with atypical symptoms.

Therefore a common management of patients with suspected GERD is offering proton inhibitors as a trial. It is usual that patients without any response to the PPI test are sent for an upper endoscopy or/and pH monitoring. With the finding of mucosal injuries at the endoscopy combined with typical symptoms of reflux the diagnosis of GERD is clear and no further examination is needed. However in patients with an examination without any mucosal abnormalities, further investigation with pH monitoring is commonly needed. In order to avoid gastric pH tracings, esophageal pH monitoring has traditionally been performed with the pH electrode positioned in the mid esophagus as the pH catheter moves significantly with breathing, during changes in body position and due to physiological shortening of the esophagus during swallowing. Thus, the positioning of the pH electrode has been chosen for practical reasons and not because it has been shown to optimize the diagnostic performance of the test. The optimal level of the pH electrode to establish the presence of abnormal acid exposure during esophageal pH monitoring is unknown. However, it has been demonstrated that the number of reflux events as well as the degree of esophageal acid exposure are significantly higher in the most distal esophagus compared with that of the level of traditionally placed pH electrodes. Also, the area of the squamocolumnar junction (SCJ) is the area were complications of GERD, such as esophagitis, strictures and premalignant metaplastic changes most commonly occur

The technical advances with the development of the wireless pH monitoring has made it possible to place the pH electrode at any desired level within the esophagus and therefore provided an unique opportunity to perform targeted pH monitoring in the area of the SCJ. This has for the first time allowed us to perform reliable studies of the acid environment in the area of the SCJ.

The aim of this thesis was to study and characterize the acid environment in the area of SCJ and to compare the diagnostic power of pH monitoring at this level with that of traditional pH monitoring. Further, to study the association between the pattern of esophageal acid exposure in the most distal esophagus and mucosal abnormalities in the area of the SCJ.

Previous studies

Symptoms and definitions of GERD

Reflux symptoms rather than complications of the disease, such as bleeding, symptoms of strictures or malignancies, are the main reason for patients to initiate contact with health services or for initiating medication. Symptoms of GERD are divided into either typical such as heartburn and regurgitations or atypical such as respiratory symptoms, cough, chest pain, dysphagia and laryngitis. However GERD may also be asymptomatic. The mechanism by which symptoms are elicited and the relationship between symptom occurrence and reflux episodes is incompletely understood. In 1958 Bernstein and Baker performed studies where they demonstrated that acid that was exposed on esophageal mucosa induced heartburn, a symptom that was diminished with treatment by acid suppressive medication (11). Symptom experience depends on individual sensitivity threshold, the refluxed volume, the composition and acid content of the refluxate, the duration of the acid exposure, which to some extent may explain variations in symptom expression. A more proximal extension of the refluxate seem more likely evoke symptoms of regurgitations while a more distal exposure is associated with heartburn. Several studies have reported a temporal association between the reflux episodes and symptoms, despite esophageal acid exposure time within normal limits (12-14). Such patients have been proposed to have hypersensitivity (14, 15). The individual differences in sensitivity to acid reflux are demonstrated by observations that GERD patients without mucosal injury (NERD) often have a higher sensitivity to heat and with a higher response to minor esophageal acid stimuli (16). An explanation to that not all patients experience symptoms may be explained by that chemoreceptors in the oesophagus of these patients with atypical symptoms may have become hypersensitive or conditioned to acid stimuli. Interestingly, individuals with BE appear to have a higher symptom threshold to acid stimuli compared with GERD patients in ambulatory pH monitoring studies (17). Studies have also shown that distension in the esophagus can induce heartburn that increase with the proximal extent of the refluxate. The sensitivity and specificity of typical symptoms of reflux in detecting GERD ranges from 6-68% and 63-95% respectively as found through studies with pH monitoring that however includes patients with more severe (erosive) disease where the pH test has a higher diagnostic precision compared with that in non erosive disease (18,

19). In patients with heartburn, esophagitis is found in 50 % of the endoscopic examinations and up to one third of asymptomatic individuals can be expected to exhibit esophagitis, which demonstrates the limitations of symptoms in the clinical diagnosis of GERD (20-25).

Several attempts have been made in defining GERD but still no universally accepted definition exists (3, 26, 27). Defining GERD is difficult because the underlying pathogenesis, mechanism behind symptoms and complications are unclear. Further, there are no specific laboratory tests or specific diagnostical tests and also, a lack of clear distinction between health and disease makes GERD problematic to define. Reflux is the involuntary movement of gastric contents into the esophagus and the result of ineffective defence mechanisms against reflux. One of the major recognized components of the refluxate is gastric acid but even in healthy individuals acid reflux occurs as a normal, physiological phenomenon. Physiological reflux occurs in all individuals, especially following meals and makes the distinction between health and disease difficult. The use of a definition of GERD based entirely on symptoms is inadequate as similar symptoms are shared with many diseases. Further, it seems reasonable to include complications of the condition, such as esophagitis, stricture, bleedings and metaplasia, to the definition of GERD. The current Montreal consensus states that GERD is a condition, which develops when the reflux of gastric contents causes troublesome symptoms and complications (3). This definition emphasizes a correlation between reflux of gastric contents and symptoms but there is an undefined threshold of severity. Very similar to the Montreal classification is the American College of Gastroenterology statement in that “GERD should be defined as symptoms or complications resulting from the reflux of gastric contents into the esophagus or beyond(28). These current definitions illustrate the heterogeneity of GERD, where the diagnosis rests on a combination of symptoms, diagnostic tests and relief of symptoms from medication.

Epidemiology and socioeconomic aspects

GERD is a chronic disease with a mean prevalence based upon weekly symptoms of heartburn and regurgitations ranging from 15 to 30 % in the western world. The prevalence in Asia is lower with reported weekly symptoms in 5-10% of the populations (2, 29). In a large Swedish population study the reported prevalence in Sweden was 16-20% based on weekly symptoms of heartburn and regurgitations (24). In the Olmstead County, USA, population study the incidence was 42.4 % based on a yearly experiencing of any episode of heartburn (1).

Patients with GERD experience considerable suffering and reports similar reduction in health related quality of life (HR-QoL) as patients with angina pectoris and congestive heart disease but they even report a well-being worse than patients with diabetes and hypertension and (30, 31). Treatment with acid supressing medication returns well-being to normal (30) but as GERD follows a chronic course, patients discontinuing treatment with acid suppression will regain symptoms (32, 33). The high prevalence of GERD, the patient suffering with accompanying socioeconomic costs and costs from complications from acid related injuries to the esophagus makes the disease clinically important.

GERD was ranked the 4 th most expensive disease of the gastrointestinal tract in the USA in 2002 and despite considerable price reductions in medication, cost from reduced labor, diagnostic tests, complications and examinations still remains high (4). In 2012 gastroesophageal reflux was rated the most common gastrointestinal diagnosis in the US (5). Visits in ambulatory care and hospital discharges in USA that were attributable to GERD increased several folded from 1990 to 2004, constituting 17.5% of all diagnoses in the digestive system. In 2004 it was estimated to account for half of the prescription costs from medication, excluding medication over-the-counter (OTC), as the most common digestive disease in ambulatory care (34). The majority of GERD patients are encountered in primary care with a diversity of symptoms that leads to diagnostic difficulties, considerable consumption of resources and health costs (35, 36).

Anatomy and histology of the gastroesophageal junction

The esophagus is normally approximately 25 cm long and passes through the diaphragmatic hiatus where after it merges with the gastric cardia. The musculature of the esophagus consists of an inner circular and an outer longitudinal muscle layer composed of striated skeletal muscle in the upper part of the esophagus and of smooth muscle distally. The gastroesophageal junction (GEJ) consists of several anatomical structures that separate the esophagus from the stomach and maintains a reflux barrier. In the distal esophagus the lower esophageal sphincter (LES) constitutes a reflux barrier, which has an internal and an external portion. The internal part consists of the intrinsic muscles of the esophagus together with the sling fibers of the proximal stomach and the external part consists of the skeletal muscle of the diaphragmatic crura, which encircles the esophagus and contributes in keeping the diaphragmatic canal closed. The distal esophagus is anchored to the crural diaphragm by the phrenoesophageal ligament (Fig 1) (37).

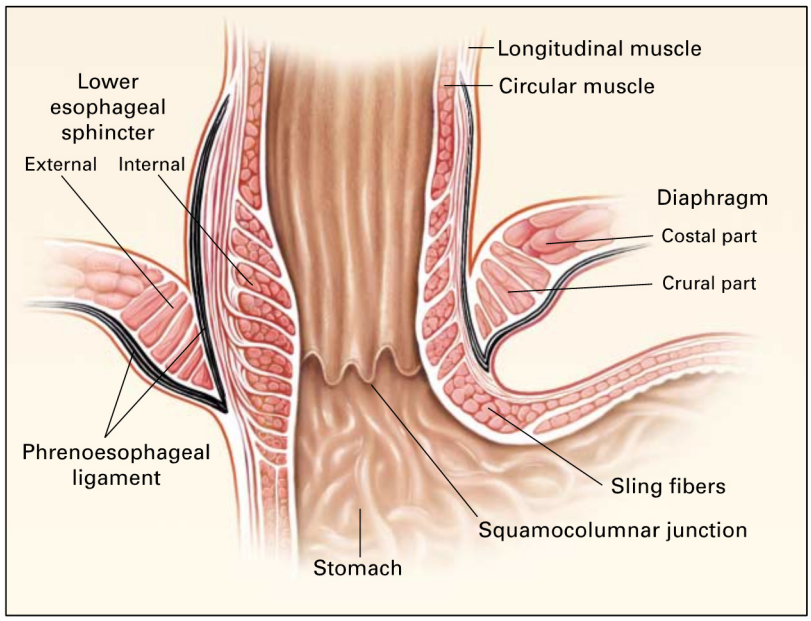


Fig 1. Anatomy of the gastroesophageal junction (reproduced by permission of the Massachusetts Medical Society)

The LES is approximately 4 cm long and endoscopically identified as a rosette formation, usually by 4-6 gastric folds, which converge in the centre of the lumen. The SCJ is identified as the demarcation between the pinkish grey coloured squamous mucosa and the salmon coloured gastric columnar epithelium. The shape of the normal SCJ is unknown, but it is commonly and without scientific evidence described as slightly irregular with minimal tongue like protrusions extending proximally into the esophagus. At the endoscopic examination, with minimal insufflation, the GEJ is identified by the proximal margin of the longitudinal gastric folds, which marks the beginning of the stomach (38). In individuals without proximal displacement of the SCJ, the location of the GEJ and the SCJ coincides (39). Overinflating during the endoscopy may lead to flattening of the mucosal gastric folds and a risk to overestimate the length between the SCJ and the longitudinal gastric folds (GEJ). Consequently there may be a risk of over diagnosing the presence of metaplastic columnar epithelium. The GEJ can be proximally displaced above the diaphragm due to presence of a hernia.

The esophagus is lined by a stratified squamous epithelium with scattered mucous glands whereas the stomach is lined by a mucinous epithelium containing either oxyntic glands or mucous glands. There are controversies regarding the normal histology at the GEJ, where some argue for the presence of a transitional zone of cardiac mucosa consisting of pure mucous glands or sometimes mixed

mucous/oxyntic glands between the anatomical gastric cardia and the esophagus (40). Others argue that the normal transition is between the squamous epithelium of the esophagus and the oxyntic mucosa of the stomach, without any segment of cardiac type mucosa, and that the presence of cardiac mucosa below the SCJ is a result of metaplasia and a consequence injury due to reflux of gastric acid (41, 42). The acid environment at the SCJ is harsh where complications from GERD such as metaplasia commonly occur. In metaplasia, the squamous mucosa is replaced by metaplastic columnar mucosa due to reflux-induced injury. The metaplastic segment may be minimal and therefore extremely difficult to identify but they may also extend well into the esophagus. The extent of the length of metaplastic mucosa depends on the degree of esophageal acid exposure (43, 44). The histology of metaplastic esophageal mucosa is normally divided into three different types. The first type is atrophic gastric fundic-type epithelium with parietal and chief cells. Second, the metaplastic mucosa may be cardiac type mucosa, which is characterized by pure mucous glands or, according to some experts, by mucous mixed by occasional oxyntic cells. Last, the metaplastic segment may be composed of a distinctive specialized columnar epithelium with a villiform surface, mucous glands and intestinal-type goblet cells. The goblet cells normally contain acid mucins, which are positive coloured by Alcian blue and periodic acidic shiff. This is in contrast to goblet cells within gastric mucosa, which typically do not contain acidic mucins. The former type of metaplasia is commonly referred to as specialized intestinal metaplasia and it is associated with an increased risk of developing esophageal adenocarcinoma and therefore often required for the diagnosis Barrett's esophagus (45, 46).

Protective mechanisms against gastroesophageal reflux

Gastroesophageal reflux occurs when gastric contents involuntarily regurgitates and transverses the lower esophageal sphincter (LES) up into the esophagus. The human body normally have several mechanisms by which reflux is prevented, which is complemented by further mechanisms to minimize symptoms and injury when reflux does occur. Theoretically, reflux disease develops when exposure to an injurious refluxate exceeds esophageal protective mechanisms. As there is no significant difference in the magnitude of the hydrochloric acid or the pepsin content of the refluxate in patients with GERD compared with healthy controls, it is likely that GERD is not a disease of production of noxious factors but rather involves the breakdown of esophageal defence mechanisms (47). Reflux is prevented by a mechanical barrier at the GEJ that consists of structural and functional components. The most important components of this barrier are the internal lower esophageal sphincter (LES) and the external sphincter mechanism consisting of the crural diaphragm and the intra-abdominal position of the LES.

Physiologically, the LES is a 3-4 cm long segment of tonically contracted smooth muscle at the distal end of the esophagus. The resting pressure remains in a closed state to prevent reflux of gastric contents into the esophagus and opens by a relaxation that coincides with a pharyngeal swallow. The LES has intrinsic myogenic tone, which is modulated by neural and hormonal mechanisms. A defective LES is typically less than 2 cm in overall length, less than 1 cm in intra abdominal length and with a pressure gradient of less than 6 mm Hg. The defective LES, an incompetent crural diaphragm and an incompetent flap valve are the major determinants permitting excessive reflux (48).

In subjects with a structurally intact LES, LES relaxations are believed to occur through mechanisms of transient lower esophageal sphincter relaxations (TLESRs). The TLESRs is a normal phenomenon during swallowing to allow food to pass from the esophagus to the stomach. It is a mechanism that relaxes the LES during swallowing and at the same time allows gastroesophageal reflux to occur. In the absence of swallowing, TLESRs also may occur as a vagally induced reflex persisting 10-60 seconds three times every hour in healthy asymptomatic individuals without causing any damage to the esophagus (49-51). TLESRs occur more frequently with gastric distension but are as frequent in healthy individuals as in GERD patients except for more often associated with reflux in symptomatic patients (52, 53).

A second component of the human mechanisms against reflux induced symptoms and mucosal injury is effective esophageal clearance (54). Clearance of the esophagus is influenced by gravity, peristalsis and salivary and esophageal submucosal gland bicarbonate secretion (55, 56). Ineffective clearance can result in an abnormal esophageal exposure to gastric juice by prolonging the reflux episode. Studies have suggested that the severity of esophagitis in GERD is associated with peristaltic dysfunction (57). The presence of hernia may also impair the natural emptying of the esophagus and the severity of esophagitis is seen to correlate with presence and size of the hernia (58). The amplitude of esophageal contractions is lower in patients with GERD compared with that of healthy asymptomatic individuals. Whether the impaired peristaltic function in patients with GERD is primary or secondary as a result of disease is unknown (59). Salivation adds to esophageal clearance by neutralizing the small amount of acid that is left after a peristaltic wave (60). With bolus acidification of the esophageal lumen, the first swallow-induced peristaltic wave clears more than 90% of the bolus volume from the lumen, but it takes several minutes before the esophageal pH has returned to normal. The restoration of esophageal pH is stepwise and associated with repeated swallows, whereby each swallow transport bicarbonate from the saliva along the esophagus to neutralize the remaining acid (60, 61). Saliva production may also be stimulated by the presence of acid in the lower esophagus, and when this it is sufficiently severe, the patient often expresses

a sensation of excessive mucous in the throat and mouth, a phenomenon clinically referred to as “water-brash” (62).

The epithelial barrier of the esophageal mucosa is another important component in the defence against reflux-induced symptoms and mucosal injury. The constituents in the refluxed gastric juice, which include acid, pepsin, bile salts and pancreatic enzymes are potentially able to permeate the mucosa and cause injury. The epithelial cellular defence composed of lipid rich intercellular space and tight junctions opposes the effect of these injurious agents. In reflux-induced injury, damage can be seen with electron microscopy as dilated intercellular spaces of the esophageal epithelia (63).

Pathophysiology of Gastroesophageal Reflux Disease

Gastroesophageal reflux disease is the result of failure of the antireflux barrier and reflux of noxious gastric juice into the esophagus, causing symptoms and/or esophageal mucosal injury. The determinants causing symptoms and mucosal injuries are the imbalance between refluxed harmful contents in the gastric juice, the defensive mechanisms and the perceptive symptom threshold in the esophagus of the individual. Even with a compromised defensive mechanisms symptoms and complications does not inevitably occur and probably because of a variable or individual susceptibility to exposure of the esophagus. The main components of the gastric juice are hydrochloric acid, bile acid and digestive enzymes intended for the digestion of food. Many components in the gastric juice are capable of damaging the mucosa but the foremost harmful component to the esophageal epithelium is hydrochloric acid shown to induce heartburn when instilled on esophageal mucosa (11) and confirmed injurious by the healing of esophageal mucosal injuries from acid suppressive therapy (64). Reflux patients with higher acid exposure have a confirmed greater risk of esophageal mucosal injury (65). The digestive protein, pepsinogen is activated into the proteolytic active pepsin, with a higher activity at lower pH, acting synergistically with hydrochloric acid causing esophagitis (66). The significance of trypsin in contributing to mucosal injury is probably minor because it is inactive at acidic pH. Free oyxygen and nitrous radicals has also been suggested contributing to damage. Esophageal mucosal injury is more severe with the combination of both reflux of hydrochloric acid and bile acid, which at pH<4 commonly co-exist in 70-90%. The aggressive effect of bile acid is pH dependent to penetrate the mucosa and exert intracellular damage, disruption on the cell membrane and tight junctions but the contribution of bile acids in reflux injuries is incompletely understood in vivo (67).

The role of Helicobacter Pylori (HP) in GERD is not clear although studies indicate HP infection of the gastric corpus decrease parietal cell mass and thus acid secretion. In contrary infection in the gastric antrum increases acid secretion via reduced negative feedback inhibition. The contribution of HP in GERD is incompletely understood and controversial but the significance is likely to be minor as other factors besides increased acid production is necessary to cause mucosal injury.

The distribution of gastric volume with the partitioning of gastric juice and food contents together with the buffering effects of food has also attracted interest in the pathogenesis of GERD. It has been shown that acid forms a layer just below the GEJ on top of the contents of the stomach termed the "acid pocket". The acid pocket has been shown to transverse the GEJ and suggested to expose the esophagus to acid for extended periods after meals but the significance of the acid pocket in the pathogenesis of GERD needs to be further studied (68, 69).

Non-Acid Reflux

Reflux of gastric contents may be acidic due to a high concentration of hydrochloric acid but may also be non-acidic. Acid reflux is objectively measured based on concentration of hydrogen ions with either the traditional catheter based pH sensor or with the wireless pH sensor but non-acidic reflux may presently only be monitored by the impedance technique. It has also been recognized that bile reflux commonly coexist with acid reflux and may cause esophagitis, demonstrated in patients with clinical GERD and endoscopic mucosal injury but with normal pH monitoring, and that acid reflux and duodenal juice potentiate esophageal mucosal damage (70-72). In Ambulatory pH monitoring there are patients with a normal pH test but who have weak acidic reflux with a clear association between reflux events to symptoms. These patients have been argued to suffer from weak acidic or non-acid reflux. The multichannel intraluminal impedance (MII) monitoring technique was introduced in the 1991 to address the limitations of the esophageal pH monitoring in detecting non-acidic reflux and the MII is also capable of detecting directional flow of refluxed contents. The primary intention with the impedance technique was to identify patients with clinical suspected GERD and negative pH monitoring (73). The importance of non-acidic reflux is probably minor since healthy controls and GERD patients with and without mucosal injury seem to have similar prevalence of weak acidic (pH4-7) reflux and it is hard to draw any major conclusion as there also is a large variation in the prevalence of weak acid reflux between studies of GERD patients and healthy (70-73). MII-pH was expected to identify the patients with normal acid reflux but with non-acid reflux. These patients are infrequently off therapy as abnormal acid reflux exists in combination with non acid reflux (74).

Complications of reflux disease

Complications of reflux are due to mucosal damage caused by the injurious effects of gastric juice. The most common complication of GERD is esophagitis. The prevalence of esophagitis reported in Sweden is 15.5 % and between 25-30% of patients with typical symptoms of GERD undergoing an endoscopy have esophagitis (24). Mucosal injuries in esophagitis is graded according to the Los Angeles classification divided into grades of severity based on the extent of injury affecting the mucosal endoscopic appearance (LAC) (75). Severe esophagitis may progress to ulcerations, bleedings and strictures, obstructing intake of food, which possibly may need dilatation of the stricture (76). Rarely, fatal complications from GERD such as hemorragic esophagitis, aspiration pneumonia and esophageal perforation occur (77). A most serious complication to reflux disease is adenocarcinoma of the esophagus, which has shown a rapid incline in incidence over the past decades (8, 78-80), and that is strongly associated with Barrett´s esophagus (BE). BE is a condition in which the normal squamous epithelium of the esophagus is replaced by metaplastic columnar mucosa secondary to reflux induced injury. It is diagnosed in the presence of columnar mucosa during on endoscopy and the presence of columnar epithelium with specialized intestinal metaplasi, which is characterized by the presence of acidic goblet cells, on histologic examination of biopsies of the columnar lined segment. BE has a 30-125 % risk in developing adenocarcinoma compared with the normal population and is also closely associated with gastroesophageal reflux (81-83). The incidence of BE varies but it is estimated that approximately 10% of patients with GERD will develop BE (84-86). In a report from 1993, the incidence of esophageal carcinomas among men in Sweden increased from 0.5 to 1.1 per 100 000 per year over a 25-year period but the annual risk incidence for malignant transformation of BE is appreciated to 0.5 % (87, 88). A disturbing fact is that 25 % of patients with BE and 40 % of all patients with adenocarcinoma are asymptomatic or report only minor symptoms of reflux (10, 21). The Mortality of Barrett´s adenocarcinoma depends on TNM staging of the disease with 90 % 5 year survival rate for stage I, 56% stage II, 15 % stage III and 0 % at stage IV. The overall survival rate is reported to approximately 50% (89). The risk of development of adenocarcinoma has been shown to be correlated to the length of the metaplastic segment, which is a reason for some of the arbitrary divisions in length of BE (90, 91). Other less serious complications in GERD include dental erosions, leading to erosive damage with subsequent extensive restoration of oral health (92, 93), laryngitis, ulcers and granuloma of the vocal folds from laryngopharyngeal reflux (94).

Treatment of gastroesophageal reflux disease

The treatment objective in GERD is primarily symptom relief and prevention of complications and secondary to prevent relapse. Treatment offered depends largely on the severity of the disease and may be of increasing intensity or step wise increasing measures or a combination of methods. Usually, as a first recommendation against reflux symptoms the patient is offered life style modifications such as weight loss when overweight or gained weight, night time head elevation and to avoid food before bedtime in case of nocturnal GERD. An increase in BMI may initiate reflux symptoms and weight loss is associated with a reduction of reflux symptoms (95-97). Another common recommendation against reflux symptoms is to avoid food that trigger reflux, such as chocolate, food containing acidic contents, alcohol, caffeine and spicy food, but no studies have shown clinical improvement of symptoms or complications by the cessation various food components. Smoking reduces LES pressure, promotes strain induced reflux and prolongs acid clearance through decreased salivation but cessation of smoking is likely only beneficial as a part of other treatment measures (98). Medical treatment constituted of antacids and H2 antagonists before the PPI became available but the effectiveness and price reductions of the PPIs has made them the preferred prescription and OTC treatment. Medication affecting acid production does not lessen reflux but reduce acid content in the refluxed gastric juice. Medical treatment can provide symptom relief but treatment of reflux disease should also include healing of complications, such as erosive esophagitis, which most effectively is offered by PPIs demonstrated by the quicker symptom relief and healing rate in reflux esophagitis (64, 99). Although acid suppressive therapy heals most complications and relieves symptoms from acid reflux in patients, GERD is chronic and the majority of the patients will have symptom relapse or recurrence of complications without continuous PPI maintaining esophageal mucosal integrity (32, 100). PPI has a very modest treatment response in symptomatic patients without mucosal injuries, who represent the majority of patients with GERD, compared with patients with esophagitis. As 40% of gastroesophageal reflux patients without mucosal injury do not respond to acid suppressive therapy it is a challenge to identify these patients that truly suffer from acid reflux related disorders (101, 102).

Alternative methods most commonly performed in severe reflux disease include endoscopic therapy and anti-reflux surgery, which intends to restore the defect mechanical barrier and a successful surgical therapy may prevent all types of reflux up into the esophagus in contrast to acid suppressive therapy. The severity of esophageal mucosal injury in GERD patients increases with the progressive loss of LES function and surgery restores the anti-reflux barrier by reducing hiatal hernia, increasing the LES pressure and restoring the intra abdominal part of the

esophagus.(65). The most common surgical technique is to mobilize fundus to envelope the esophagus and approximating the diaphragmatic crura. Success in symptom relief and healing of erosive esophagitis with laparoscopic therapy is > 95% and 90% respectively. Success depends on patient selection, which is based on typical symptoms of reflux, clinical response to treatment with PPI, presence of erosive esophagitis, defect LES and abnormal 24 h esophageal pH monitoring(103). Complications are uncommon and side effects include a less than 10% risk of persistent dysphagia, increased flatulence, occasional bloating and early satiety (65, 104). Fatal or life threatening complications and overall mortality was previously 0.8% and 0.3% respectively, which have been reduced with the laparoscopic surgical technique (105, 106). The long-term symptomatic relief effect in laparoscopic fundoplication is on a 10-year basis 90% and a 20-year basis 80%(107).

An alternative to surgery or long-term acid reducing therapy is endoscopic therapy, such as application of controlled radiofrequency energy (RF) to the lower esophageal sphincter region (Stretta) inducing collagen contraction, which successfully reduces symptoms in around 20 to 30%. Transoral incisionless fundoplication involves restoration of the incompetent antireflux barrier by longitudinal and rotational movement of the gastric fundus around the lower esophagus extending proximally 3.5 cm above the Z-line resulting 40 % persistent 24 months relief and reduction in symptoms in 25-50 % of the patients. Other techniques such as injection of bulk agents or other endoscopic suturing techniques have been abandoned due to adverse effects or lack of long-term efficacy (108). The three main methods (Stretta, Endocinch, Enteryx) have similar positive effect on improvement of symptoms, QOL and PPI use but still esophageal acid exposure remains similar as previously before therapy, why it seems appropriate to consider these methods exclusively in patients with persistent symptoms and uncomplicated GERD without relief from other therapy (109).

Diagnostical tests in the diagnosis of GERD

The diagnosis of a disease in clinical practice rests largely in discriminating symptoms characteristical for the disease often combined with laboratory blood tests, radiologic methods and physical examinations. GERD can be verified by different diagnostic methods and the most common examination is endoscopy where the upper gastrointestinal tract is examined with a flexible video endoscope.

Endoscopy

Endoscopy is a highly valuable instrument often performed in patients when complications of GERD are suspected, if unsatisfactory result of acid suppressive medication, in differential diagnostic consideration, as a control of healing after therapy or in considerations to anti-reflux surgery. In the endoscopic examination the anatomy and the macroscopic appearance of the upper gastrointestinal tract are visualized, biopsies may be obtained and therapy performed when needed. Complications from acid injuries and HP infection such as atrophy, gastritis, strictures, peptic ulcers, esophagitis, cancer and metaplasia can be identified at the endoscopy. The endoscopic finding of esophagitis or BE confirms the diagnosis of GERD and no further investigation is needed (110). However, in the majority, constituting two thirds of the patients with GERD, the endoscopic examination is normal, which often necessitates further investigation (111). As not all patients with GERD can be identified with endoscopy, other medical conditions may elicit similar symptoms and antireflux therapy at times result in an unsatisfactory treatment response, there is need for additional objective methods to evaluate reflux disease in the management of patients with GERD.

Manometry

Manometry is performed mainly to identify and evaluate the lower esophageal sphincter in the diagnosis of motility disorders, such as achalasia, scleroderma, and in identifying the LES for the positioning of the pH catheter in ambulatory pH monitoring. Preceding the pH monitoring, a full nasal or transoral manometry is performed to characterize the LES after an overnight fast using a stationary pull-through technique with an 8-channel catheter (112). The LES resting pressure is defined at the respiratory inversion point. The overall length, abdominal length of the LES and the LES resting pressure is determined by the mean of five recordings. For the placement of the pH catheter the distance between the incisors or the nostril to the manometrically defined upper border of the LES is used. In investigation of suspected esophageal motility disorders the patient swallows 5 ml water 10 times with 30 seconds intervals in between. The peristalsis and amplitude of the contraction is evaluated and calculated from the values of the mean.

Barium swallow

Radiologically, barium swallow is used to visualize the esophagus, delayed esophageal emptying, presence of reflux into the esophagus, hernia, stricture and to some extent to detect mucosal injury. Previously the single contrast method was used and presently the used method is the double contrast, which increases the diagnostic value of the test. In comparison with endoscopy, the diagnostic accuracy is considerably lower even in patients with moderate esophagitis (113).

The primary use is to visualize hernia, strictures and motility disorders together with esophageal deformation. When endoscopy became available the barium swallow as a diagnostic method in the diagnosis of reflux disease has much disappeared and cannot be recommended except for in selected cases, in the evaluation for surgery, in the diagnosis of achalasia and systemic sclerosis.

Capsule endoscopy

Capsule endoscopy is performed through swallowing a capsule containing a camera, which takes multiple pictures, a transmitter and a battery. Due to the fast passage of the capsule through the esophagus during the swallowing act, the camera cannot capture enough pictures for the evaluation of the esophagus, which limits the clinical usefulness. The inability for biopsies also limits the technique. Possibly with development, capsule camera endoscopy can be used in future in the evaluation of the esophagus. So far the technique also has a reported suboptimal diagnostic accuracy in the evaluation of BE, hernia and esophagitis, but possibly it can be used in patients refusing endoscopy (114, 115).

24 hour spectrophotometric esophageal bilirubin monitoring

Bilirubin from the hepatic bile ducts may be refluxed from the duodenum into the stomach and further up into the esophagus. Bilirubin can be detected by a fiberoptic probe (Bilitech 2000, Medtronic, Shoreview, MN, USA), which measures the specific light absorption of bilirubin, as a marker for duodenogastroesophageal reflux (116). A limitation to Bilitech is that with acidic pH, absorbance level is lower, which thus underestimates the amount of bile (117). Bilitech can only detect biliary reflux as there is no correlation between biliary and non-acid reflux (118).

Esophageal multichannel intraluminal impedance measuring

The impedance technique detects and measures the flow of liquids and gas through a hollow organ on the basis of the difference in electrical resistance between two positions that are different depending conductivity of the substances at the measured positions. Gas, fluid or a mixture of the two has different conductivity. This can be used to evaluate the occurrence of reflux and the type of medium such as gas, liquid or mixed liquid and gas (119). Multiple electrodes are positioned along the axial length of the impedance catheter such that the proximal extent of a reflux event can be determined. The order in which the different sensors react can be used to determine the direction of the refluxed contents. Passage of liquid between two sensors causes a drop in impedance value of more than 50% from

baseline and conversely an increase at 50% above baseline indicates gaseous content. The obvious advantage of multichannel intraluminal impedance (MII) over pH monitoring is that it can detect any type of reflux and the direction of it. Impedance monitoring is not able to detect either the acid content or volume of the intraluminal contents. Therefore, a pH electrode is typically integrated into the recording assembly. A pH drop combined with a simultaneous impedance change identifies an acid reflux episode. As the impedance technique requires the use of a catheter, the limitations of the technique is similar to that of catheter-based pH monitoring, which is patient discomfort, disturbance of normal daily activities, possibly disturbance of esophageal motility by the catheter. Further, technique does not allow for adequate monitoring of reflux in the area of the GEJ as the catheter moves significantly with breathing, swallow-induced esophageal shortening and with changes in body position. The usefulness of MII in clinical practice is uncertain, as isolated alkaline reflux is a rare phenomena and presence of weak acidic reflux in patients with moderate to severe esophagitis is similar to that of healthy individuals. Other limitations to the MII are that it has not shown useful to predict treatment response or outcome after anti reflux surgery (120, 121). The impedance technique has led to a deeper understanding of GERD and it is promising that there now is a way to appreciate non-acid reflux and maybe the impedance sensor and the technique may be further developed to be of more clinical value (119).

Acid suppression test

Acid suppressive therapy is commonly tested in patients without symptoms indicative of complications. A treatment response of symptom relief is usually taken for a correct diagnosis (122). Using the PPI test for 14 days has shown a similar diagnostic accuracy such as pH monitoring with a sensitivity and specificity of 80% and 74% respectively (123). In patients with NCCP where erosive disease is rare the clinical value of endoscopy is limited and PPI may be used as a diagnostic test (124). Although the 24 h pH test is the strongest predictor of outcome in the evaluation for anti reflux surgery, the acid suppressive medication test also has a proven clinical value in the detecting suspected GERD but the placebo effect has to be considered (103, 125).

Esophageal pH monitoring

The most objective diagnostic test used for quantifying esophageal acid exposure is ambulatory pH monitoring. Historically the first attempts to study gastric juice were made in patients with heartburn in 1884 with gelatin-coated sponges lowered into the esophagus that were retrieved containing acid (126). Later gastric juice

was collected from an esophagitis patient with a tube, which contained acid and pepsin. In 1958 the first acid reflux measurements were made by Tuttle and Grossman with gastric PH metry equipment placing the probe in the esophagus to measure intra esophageal pH. Prolonged pH monitoring was first initiated by Spencer in 1969 and in 1974 Johnson and Demeester published the first normal values for 24 h pH monitoring in healthy controls and GERD patients (127, 128). The early setting needed the patient to be hospitalized and not until the 1980s, ambulatory pH monitoring became possible with portable equipment. Using telemetry for sending pH data via radio signal has been available since the 1980s but the equipment could not be secured in the esophagus and so the pH capsule needed to be swallowed and attached to the patient's mouth by a thread. The modern esophageal pH system consists of catheter carrying a glass or antimony sensor, the reference electrode and a data receiver. A reference electrode is either placed externally on the patients skin or is inbuilt into the pH catheter. The internal reference system is considered superior compared with the external reference electrode, which is disturbed by the skin contact during the pH monitoring and may result in artefact pH readings due to difference in mucosal potential between skin and esophageal mucosa (129). The ideal pH sensor should be small, inexpensive, and disposable and technically have a rapid response time between pH 1-7. It should preferably be unaffected by room temperature, have fast response to changes in pH and without pH drift during the monitored time. The pH sensor of today is a compromise from these requirements. Glass electrodes are electrochemically superior to antimony electrodes with quicker response, less drift and a better linear response but on the down side they are larger, more expensive. The glass electrode have a similar performance as the antimony electrode when used in intra esophageal pH monitoring with pH less than 4 (130).

A different pH sensor is the ion sensitive field effect transistor, which has a temperature stable calibration and even more accurately detect acid exposure than the antimony and glass electrodes in studies, but it has not been implemented in clinical use (131). Antimony pH electrodes have been considered adequate for clinical use in pH monitoring with an acceptable technical performance unlikely to affect the evaluation of the pH test (132). In pH monitoring the hydrogen ion concentration is measured and the negative logarithm of the hydrogen ion concentration defines the pH value ($pH= +\log 1/ [H^+]$). A logarithmic measurement translates into that the pH 1 will contain 10 times the hydrogen concentration of a solution with pH 2. The electrical potential difference generated by a concentration gradient of hydrogen ions, between two electrodes is extrapolated to a pH value.

Catheter based pH monitoring

The conventional catheter-based pH monitoring system has a flexible catheter with one or more pH sensors and a data logger. The catheter is introduced trans nasally and positioned in the esophagus based on manometry with the proximal end secured to the patient´s nose with adhesive tape to restrict movement. The catheter is passed through the nose down the pharynx and positioned with the pH sensor in the distal esophagus. The data logger is carried around the patient's waist during the study and the system samples pH data every 4-5 seconds depending on the manufacturer of the catheter-system. Ambulatory catheter-based pH monitoring is usually performed during 24-hours in catheter based pH systems, which allows for assessment of esophageal acid exposure with all activities during a complete circadian period. The patient is encouraged to engage in all normal activities and are instructed to report meal periods, supine, upright position and notes the presence of symptoms in the monitored time period. In some centers the patients are instructed to avoid tobacco, acidic food. Usually, pH monitoring is carried out whilst the patient is off acid production inhibiting drugs. A potential limitation in the on-therapy testing is that the reduction in gastric acidity converts strong acid to weak acid or to non-acid reflux episodes that are not detected by pH monitoring. The pH monitoring system only measures acid that reaches the level of the sensor in the esophagus so the positioning of the pH sensor should ideally be placed at the position of interest. Studies using pH catheter with multiple pH sensors at different levels has proven acid exposure is higher distally compared with proximally in the esophagus, which suggests that acid exposure is decreasing with increased distance from the LES (133-135). To avoid gastric pH tracings, which may occur due to movement of the pH catheter during changes of body position and with the natural shortening of the esophagus during swallowing, the pH sensor is by convention positioned 5 cm above the upper margin of the manometrically defined LES. After the monitoring period the pH data is down loaded into a computer for analysis.

Wireless pH monitoring

The most significant recent technical advance in pH monitoring is the incorporation of the antimony electrode into a wireless capsule that transmits pH data to an external receiver via radiofrequency telemetry (433 MHz). The data sampling occurs at 6-s intervals with the wireless pH capsule (Bravo^{TM} system, Medtronic, Minneapolis, MN). The sampling is marginally slower than the slim-line pH system at 4 to 5 seconds. The wireless system consists of capsule attached to the end of a delivery catheter and a pager sized receiver carried by the waist of the patient. The delivery catheter is 6 F and 80 cm length equipped with a release handle orally and the capsule at the distal end. The capsule is 6 x 5.5x 25 mm and houses an antimony pH electrode, a reference electrode, an internal battery, a well

and a transmitter all encapsulated in epoxy. With a vacuum created from 45 seconds continuous suction at >510 mm Hg the mucosa is captured into a 4 x 3.5 mm well and secured by a stainless pin released from the handle. The pH electrode is calibrated in a buffer solution at pH 1.0 and 7.0 and then activated by a magnetic switch before use. The pH capsule continuously transmits an own unique identity code every 12 seconds during activation that allows more than one pH capsule receiver system to be used simultaneously. The wireless pH capsule system uses the unregulated 433 MHz radiofrequency, which potentially could be interfered with other wireless systems and devices using the same radiofrequency. Interruption of the radio signal is although more common with the receiver being beyond range of the transmitter, such as when the patient is showering or performs other activities and detaches the receiver (136). The major advantage of wireless pH monitoring is less patient discomfort and consequently higher tolerability that allows the patient to continue normal daily life activities under more physiological conditions without restrictions. Other advantages include an optional targeted consistent positioning during the test. Securing the capsule with the steel pin allows for prolonged recording periods over 48 hours or more. A minor problem is the risk premature detachment of the capsule reported in the range 1.5 to 5.6 % (136-140). An undesirable detachment of the pH capsule is easily identified on the pH tracings by the drop to gastric pH and sequent a return of pH to above 6 when it passes to the duodenum. Other side effects is foreign body sensation and chest pain, which although has not proved to be a problem necessitating the removal of the capsule. The reported normal values of esophageal acid exposure for wireless pH monitoring at the conventional 6 cm level above the SCJ in control subjects, represented by the 95th percentile, is between 4.4% and 5.3% (138, 141, 142). The reported normal values are generally higher than those previously reported for the catheter-based system(143) but both higher and lower values of catheter based and the wireless system has been reported (144-146). One explanation to the variation in normal values of esophageal acid exposure is explained by a poor selection of healthy asymptomatic patients, small study size and on a variation in the definition of disease. The relatively higher acid exposure threshold reported in healthy controls using the wireless pH system may be a consequence of less restrictive daily habits or possibly technical such as a minor thermal calibration error that exists in the pH catheter systems.

Indications for pH monitoring

The most widely used diagnostic method in the evaluation of gastroesophageal reflux in GERD is by ambulatory pH monitoring, which quantifies the acid exposure to the distal esophagus. The specificity and sensitivity of the method is

87-96% and 97-100% respectively (143, 146) in patients with severe reflux disease, characterized by erosive esophagitis and defective lower esophageal sphincter. PH monitoring verifying abnormal esophageal acid exposure is generally not needed in patients with endoscopic evidence of reflux induced esophageal mucosal injury and there is generally no indication for reflux testing in the majority of patients with GERD who get adequate symptom relief and are content with medical therapy(147). However, in patients with atypical symptoms and symptomatic patients without mucosal injuries there is need for further investigation. As one third of the patients without mucosal injuries can be identified with an abnormal acid exposure there is a clinical value in performing pH monitoring in patients with persistent reflux symptoms refractory to PPI therapy (19). Patients with dyspepsia cannot satisfactory be identified with PPI trial due to inadequate sensitivity (78%) and specificity (54%) and therefore pH monitoring can be considered in endoscopy negative dyspeptic patients with persisting symptoms (21). As not all patients with acid related reflux respond medical therapy and have persisting atypical or typical symptoms, there is an indication for performing pH monitoring in such patients. PH monitoring is also helpful before surgery in patients with esophageal mucosal injury as the presence of an abnormal 24-hour pH score and an association between symptoms and reflux events has been shown to be the strongest predictor of a good surgical outcome(125). One of the most common uses of pH monitoring is in the evaluation of patients with persisting typical or atypical reflux symptoms despite adequate medical or surgical therapy

Interpretation of pH tracings

The pH range in the foregut is between pH 1 to pH 8, which is maintained by gastric acid secretion to a pH 1 between 2 and rarely above 3 whereas the duodenal pH lies in the range of 6 to 8. The pH in the esophagus is kept under normal conditions in the range between pH 5 to 7 by swallowed saliva and esophageal bicarbonate secretion(148). In most centres, based on several observations such as that heartburn is initiated below pH 4 (149) and that pepsin is inactivated above this level (150, 151), the most common level chosen for detection of abnormal acid exposure is a pH below 4. Studies on normal values for 24 h ambulatory pH monitoring have shown that in healthy individuals pH was above 4 in 98.5% of the monitored time (146). The normal upper time limits varies depending the study population and method of pH monitoring used from 3.4 % to 5.5% in the catheter based 24 h method and between 4.4% to 5.4% in the capsule based 48 h method(127, 138, 141-143, 146, 152). Esophageal pH tracings show presence of acid and pattern of reflux in the esophagus but not volume. The generated graphical pH report can be used to study the characteristics and pattern of reflux distributed in time units. The basis for interpretation is a comparison of the result from the performed pH recording with defined normal values. The

pattern of reflux provides valuable information that can be used to identify physiological acid reflux, seen in healthy individuals, and abnormal acid reflux, where the increase in severity is characterized by increasing intensity from upright position to both the upright and supine positions. The duration and the frequency of reflux episodes increase with the severity of acid reflux disease. Parameters routinely calculated by the software of the pH system include the frequency and duration of reflux events, the total number of reflux episodes, those lasting longer than 5 minutes and the duration of the longest episode together with a graphical pH tracing and symptom-reflux correlations. The parameters are presented for the entire study and for the upright, supine and postprandial periods of the recording period.

An abnormal acid exposure is defined as a value greater than the 95th percentile in normal controls that pH is less than 4, commonly defined by above 4% of the monitored time with catheter based systems. A detailed evaluation of the pH tracing is important to recognize and exclude monitoring artefacts and to evaluate the temporal relationship between symptoms experienced and recorded reflux episodes. Esophageal acid exposure is affected by body position and meals and therefore the report are divided into periods of upright and supine position and meal periods. The manual assessments of the pH tracings help detect normal physiological acid reflux, typically characterized by short, rapidly cleared reflux episodes occurring in the upright after meals. Another method in analysing and evaluating acid exposure data is the cumulative DeMeester score that includes total time with pH less than 4, the longest reflux episode, number of reflux episodes with pH less than 4. An abnormal DeMeester composite score is a value larger than 14.7 (127). Esophageal pH monitoring is usually performed during 24 hour with the catheter technique but is normally performed during 48 h with wireless pH monitoring technique. Figure 4 shows 48-h pH tracings in a subject with physiologic reflux performed at the SCJ and at 6 cm above the SCJ.

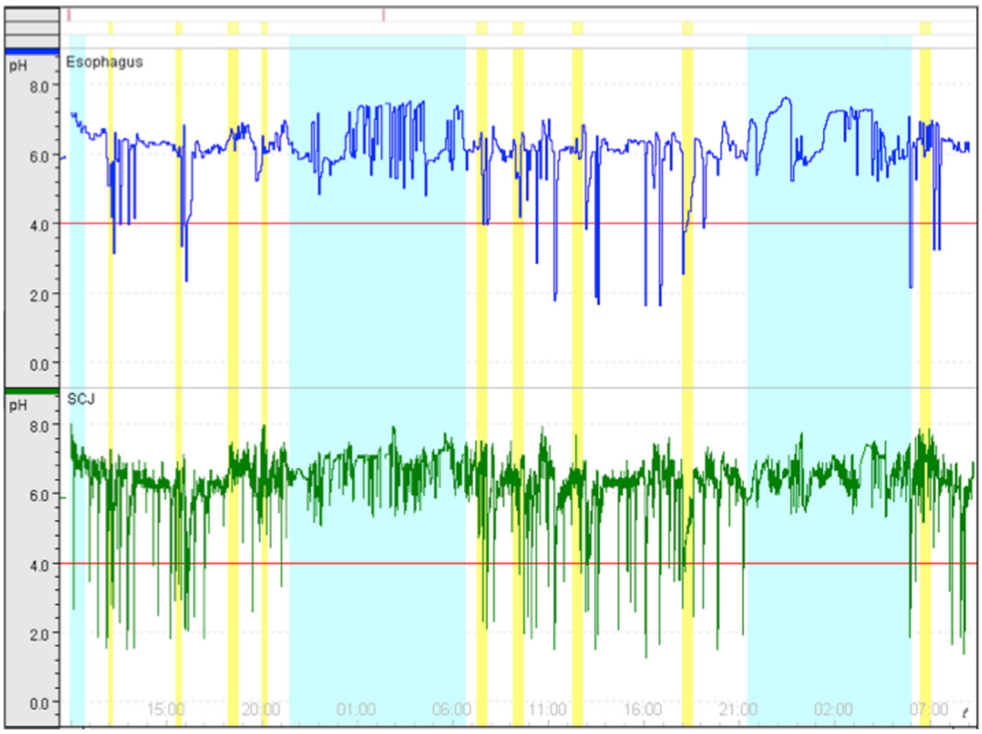


Fig 4. PH tracing with physiological reflux performed immediately above the SCJ and 6 cm above the SCJ.

Association reflux events – symptoms

Reflux symptoms are not specific for GERD, which makes it important to determine if there is a temporal association between symptoms and a reflux events. There are a few indices to assess temporal association between symptoms and a reflux event, where the most commonly used are the symptom association probability (SAP), the symptom index (SI), and the symptom sensitivity index (SSI) (153, 154). The SI is defined as the percentage of reflux episodes associated symptoms and the SSI as the percentage of symptoms associated with reflux events (155, 156). There are disadvantages with each index to consider when analysing the indexes. The SI does not take the total number of reflux episodes into account and the SSI does not include the total number of symptom events into the equation. As a consequence, the probability that SI becomes positive increases with an increasingly high number of reflux episodes and SSI is more likely to be positive when the number of symptom episodes is high. A positive symptom index score therefore necessarily does not mean that there is an association between symptoms and reflux episodes. A symptom sensitivity index (SSI) greater than 10% and a symptom index (SI) of at least 50% are normally considered positive and suggest an association between symptom and reflux. Another symptom index

is the SAP, which is a statistical method to determine the relationship between symptoms and reflux episodes. The SAP is calculated by arranging the pH-data from the entire study into consecutive 2-minute segments. For each of these segments the occurrence of a reflux event is determined, providing the total number of 2-minute segments with and without reflux. Subsequently, it is determined whether or not a reflux episode occurred in the 2-minute period before each symptom. A 2 x 2 table is constructed in which the number of 2-minute segments with and without symptoms and with and without reflux are tabulated. Using the Fisher's exact test a p-value is calculated and the SAP index is calculated as (1-p) x 100% (157). The cut off value for a positive test is often defined as SAP $\geq$ 95%. Normally the software programme of the computer calculates and supplies the data in the result.

Limitations of esophageal pH monitoring

Ambulatory pH monitoring is a valuable tool in the management of patients with symptoms suggestive of GERD as it provides quantitative data and suggests causal relationship between symptoms and acid reflux. Catheter based pH monitoring is associated with patient discomfort due to the necessary nasopharyngeal placement of the catheter in the esophagus that makes the method not tolerated by all patients. The catheter cause discomfort, which may lead to the patient avoids normal daily activities, intake of food and drink and may also be socially embarrassing and restrict the patients to less reflux provoking habits (158). Studies of wireless pH monitoring have shown abdominal pressure increases with physical exercise and consequently also reflux, which probably decreases sensitivity of the pH monitoring with restrictive daily habits (159). Although the pH electrode is positioned in the esophagus and secured with tape to the patients nose it may still move freely in the esophagus and with physiologic shortening of the esophagus during swallowing and changes in body position a consistent position of the sensor is not guaranteed. The risk of dislocation and picking up gastric pH tracings has led that the catheter by convention ha been placed 5 cm above the manometrically defined upper border of the LES. Reliable pH data requires a pH catheter with a consistent position, which cannot be maintained with a free movable esophageal pH catheter. Obviously using a catheter based pH monitoring system the pH recordings obtained may originate from different positions in the esophagus The wireless pH monitoring system in contrary allows for a fixed consistent position with the stainless pin securing the capsule at any desired level unaffected by esophageal shortening or body movement. It also offers prolonged pH monitoring recording time and less patient discomfort with less restrictive habits providing recordings under more physiological conditions. With the wireless pH monitoring the pH sensor is still placed by convention 5 cm above the LES, corresponding to 6 cm in the catheter technique, under endoscopic guidance, which offers a verification of the position. The wire less pH monitoring is preferred and better

tolerated by the patients than the catheter based system(160). However, the diagnostic precision of the pH test has not improved significantly, which has been argued to depend on patient selection with less severe disease(141). As it is well known that the degree of acid exposure decreases with the proximal distance from the LES and the area with most frequent complications is near the GEJ it is possible that an explanation lies in the choice of positioning the pH capsule at the conventional level, instead of closer to the SCJ, which could lead to underestimation of acid close to the SCJ.

Limitations to the wireless pH monitoring system include premature detachment of the capsule, which is seen in between 1.5-4 % of the patients(136, 138, 140, 161), lost radio signal transmission, or interference of the radio signal from technical devices using the same 433 Mhz radio frequenzy.

It is important to understand that the discriminatory power of the pH test increases with the severity of reflux disease and that sensitivity and specificity reported only reflects differences in the populations tested (141). The sensitivity and specificity of catheter-based pH monitoring at 87-96% and 97-100%, respectively dates back 30 years and are based on patients with defective LES and severe reflux disease with complications (143, 146). More recent studies with the wireless technique in patients with typical reflux symptoms and esophagitis report sensitivity at 76-78% and specificity at 93-95% but in patients without mucosal injuries the sensitivity drops down to 36-42% (138, 141). Unfortunately the diagnostic precision of pH monitoring is a limited by a much lower sensitivity at 60% and specificity around 85-90 % in patients without mucosal injuries, which substantially limits the clinical value of the pH test and means that in the majority of reflux patients without mucosal abnormalities and normal pH recordings, the possibility of GERD still exists (111, 162, 163). In this more common patient category with symptoms suggestive of reflux disease and uncomplicated disease, the relatively low diagnostic precision and the pH test is of limited clinical value. It is also important to apprehend the existence of patients with symptom-associated reflux without abnormal acid exposure, which cannot be identified with the means of the pH test (12, 164).

Aims of the study

This thesis aims to study and characterize the acid environment at the GEJ in the area of the SCJ esophagus and to compare the diagnostic precision of pH monitoring performed immediately above the SCJ with that observed at the conventional level in the esophagus. Further, to study the association between the pattern and degree of esophageal acid exposure and mucosal abnormalities in the most distal esophagus

The following aims were addressed in the original papers:

I. To study the acid environment in the most distal esophagus at the SCJ and to compare that with the observed at the conventional esophageal pH monitoring level. To compare the discriminatory power of wireless pH monitoring performed immediately above the SCJ with that performed at the conventional level, 6 cm above the SCJ by simultaneous pH recordings.

II. To confirm the results of the diagnostic accuracy in pH monitoring performed immediately above the SCJ obtained in study I and to study the influence of symptom analysis on the diagnostic performance of the pH test with a larger inclusion of individuals.

III. To evaluate the importance of the acid pocket in the pathogenesis of GERD by characterizing the pattern of acid reflux in the most distal esophagus with 48-h pH monitoring during the postprandial (90 min) periods and compare that with upright, preprandial periods in healthy individuals and patients with GERD.

IV. To study the prevalence of intestinal metaplasia in the normal SCJ and to correlate that with gastroesophageal reflux and/or with HP infection in a large group of asymptomatic volunteers and patients with symptoms suggestive of GERD.

V. To study the endoscopic appearance of the squamocolumnar junction and correlating the appearance to clinical characteristics, degree and pattern of acid reflux in the most distal esophagus in large groups of asymptomatic volunteers and patients with symptoms suggestive of GERD.

Study Population

Paper	Participants	Eligeble (n)	Excluded (n)	Included (n)	Monitored Level
I	Patients typical Asymptomatic volunteers	70(p) 55(c)	8(p) 6(c)	62(p) 49(c)	2 level (6 cm Z) simultaneous
II	Patients typical Asymptomatic volunteers	128 (p) 55(c)	46 6	82 49	2 level (6 cm Z) simultaneous
III	Patients typical Asymtomatic volunteers	83(p) 55(c)	8 5	75 50	Z
IV	Patients typical/atypical Asymptomatic volunteers	157(p) 55 (c)	8+ 35 5+15	114 35	Z
V	Patients typical/atypical Asymptomatic Volunteers	157 (p) 55 (c)	8 5	149 50	Z

P = Patients

C= Control

Paper I

From October 2005 to October 2006 patients between 20-70 years of age referred for esophageal pH monitoring to Lund University Hospital, department of Surgery at the Endoscopy unit were recruited. The recruitment was enhanced with patients with esophagitis identified in the hospital database, advertisement within the hospital and in the local newspaper. Fifty-five asymptomatic volunteers between 20-70 in age were included from a previous study with the objective to investigate the feasibility, safety and normal values of wireless pH monitoring as control (136). Seventy patients with typical symptoms of reflux, such as heartburn and regurgitation, more than twice weekly for at least 6 months with treatment relief from PPI met the inclusion criteria. Subjects that underwent a successful upper

endoscopy and dual simultaneous 48 h pH monitoring immediately above the SCJ and at the traditional 6 cm level above the SCJ were eligible for the study, which included 62 patients and 49 asymptomatic volunteers.

Paper II

Between October 2005 and April 2010 128 patients with symptom suggestive of GERD were recruited. The inclusion criteria were similar as in study I. Forty-six patients of the patients had atypical symptoms or inadequate response to PPI therapy and were therefore excluded. Sixty-two of the patients had participated in previous studies. Only patients with successful upper endoscopy and dual positional simultaneous 48 h pH monitoring as in study I participated in the analysis. A total of 82 patients and 49 asymptomatic volunteers met the criteria.

Paper III

Seventy-five patients with typical reflux symptoms and 55 healthy volunteers recruited from October 2005 until October 2006 met the criteria of the study. Sixty-two patients originated from study I and another 13 patients were recruited until April 2008. Data from patients and healthy asymptomatic volunteers having a technically successful pH monitoring with the pH capsule immediately above the SCJ with at least 36 h data were analysed.

Paper IV

Between October 2005 and April 2010, a total of 157 patients with typical and atypical symptoms of GERD and 55 asymptomatic healthy volunteers, from previous studies were recruited. Subjects that underwent upper endoscopy and a successful 48 h pH monitoring immediately above the SCJ were included. Eight patients and 5 healthy volunteers were exluded for technical reasons. The patient population consisted of 94 patients with typical symptoms of reflux, such as heartburn and regurgitation, and 55 patients with atypical symptoms of reflux, such as respiratory symptoms, chest pain and epigastric pain. After the exclusion of subjects for technical reasons another 35 of the patients and 15 of the healthy volunteers were excluded due to the objective was to study individuals with an endoscopically normal SCJ. Thus the analysis included 114 patients and 35 asymptomatic healthy volunteers.

Paper V

The recruitment consisted of the same participants as in study IV. The analysis consisted of 149 patients with typical and atypical symptoms suggestive of GERD together with 50 asymptomatic healthy volunteers that underwent upper endoscopy and a successful 48 h pH monitoring immediately above the SCJ.

Methods

Study design

Between October 2005 and April 2010, a total of 157 patients with both typical and atypical symptoms suggestive of GERD and 55 asymptomatic volunteers were included in these prospective studies. The patients were recruited by advertisement within the hospital and the local newspaper and by referral to the endoscopy unit at the Department of Surgery, Lund University Hospital. The inclusion was complemented with an on going recruitment of additional patients diagnosed with esophagitis identified through the hospitals outpatient database and contacted by mail for participation. All subjects underwent an upper endoscopy and dual ambulatory wireless (BRAVO capsule) 48-hour esophageal pH monitoring at the Endoscopy unit, Lund University Hospital. Only patients with technically successful pH recordings were included. Patients between the ages of 20 to 70 years without a history of previous gastric or esophageal surgery, severe cardiopulmonary disease, or symptoms suggestive of esophageal motor disorders were considered eligible for the study. Coagulopathy, severe esophagitis/stricture, long segments of esophageal columnar lining, portal hypertension and pacemaker were considered contraindications to the wireless pH system and were thus excluded from participation. Only patients with esophagitis corresponding to no higher than grade A and B according to the LAC were included in the study analyses. Treatment with proton pump inhibitors was discontinued 10 days before the test but antacids were allowed until 24-hours prior to the study. Unrestricted amounts of food were allowed but study individuals were asked to avoid acidic food products and alcohol and to keep tobacco consumption as low as possible during the monitoring period. As this was an on going project where patients were being included during the course of the studies, more patients were available for analysis in the more recent papers.

The presence of erosive esophagitis, hiatal hernia, columnar lined esophagus was documented and the presence of Helicobacter Pylori analysed with histologic examination from two biopsies obtained from the gastric antrum with the addition of faecal HP antigen test. IM was analysed with histologic examination from biopsies obtained in the gastric antrum and at the SCJ. The area of the GEJ and the SCJ were carefully studied, video documented for the purpose of re-evaluation in

uncertain cases. Measurements were made in relation to an open pair of biopsy forceps.

Paper I, II

Patients and asymptomatic volunteers underwent upper GI endoscopy with placement of two wireless capsules. One pH capsule was secured immediately above the SCJ and one pH capsule at the conventional level for pH monitoring 6 cm above the SCJ for simultaneous ambulatory esophageal pH monitoring. The duration of the pH monitoring was 48 h. Paper II had larger inclusion of patients and the evaluation of symptom index analysis, SI, SSI and SAP, was added as an individual marker of GERD and also combined with the pH monitoring data. The discriminative power of wireless pH test was evaluated and sensitivity, specificity and thresholds for esophageal acid exposure were analysed using receiver operating characteristics (ROC) curves. The performance of wireless 48 h pH recording immediately performed just above the SCJ and at the 6 cm level was compared.

Paper III

Patients and asymptomatic healthy volunteers underwent upper GI endoscopy with the placement of two wireless capsules. In this study only pH recordings from the pH capsule immediately above the SCJ was used. Patients with typical symptoms of reflux participated in the study and pH data of no less than 36 h monitoring were used. A reflux event was defined as a drop in pH to lower than 4. The computer software calculated characteristics of esophageal acid exposure but the nadir pH and the duration of each reflux event were measured manually on the computerized pH tracings of all participants. An acid pocket has been observed up to 90 minutes after meals and so postprandial reflux was defined as a reflux episode occurring within 90 minutes following a meal period. In this study reflux episodes in upright posture not occurring during meals, prandial, or after meals, postprandial, were defined as preprandial reflux. Preprandial reflux events were compared with postprandial reflux events.

Paper IV, V

The study included healthy asymptomatic volunteers and patients with typical symptoms, such as heartburn and regurgitations, and patients with atypical symptoms of reflux, such as respiratory symptoms, chest pain and epigastric pain. All subjects underwent upper GI endoscopy with placement of two wireless capsules but only the data of the distal capsule immediately above the SCJ was used in the analysis. The duration of the pH study was 48 h and recordings less than 36 hours were considered unsuccessful and consequently excluded. Based on

our results from study II the acid exposure was diagnosed abnormal when the percent time with pH less than 4 was 5.7% or more of the monitored time (165).

The appearance of the SCJ was assessed according to the Z line appearance (ZAP) classification and correlated to clinical characteristics and the degree of acid exposure to the distal esophagus. In study IV two biopsies were obtained from the gastric antrum for the histologic analysis of IM and HP infection and for the analysis of intestinal metaplasia at the SCJ, 4 biopsies were obtained from the base of the SCJ for histologic evaluation. The biopsy forceps was placed with each jaw on either side of the SCJ in order to obtain both squamous and columnar mucosa. Faecal antigen test was obtained as an additional analysis for the presence of HP and the presence of hernia was noted. As the purpose of study IV was to study the presence of IM at a normal SCJ, in the study defined as either ZAP grade 0 or ZAP grade I, patients and subjects with ZAP grade II was excluded from the analysis. There were no subjects with ZAP grade III entering the analysis of both studies as long length of columnar lined mucosa was considered a contraindication to the wireless pH monitoring technique

Endoscopy

A complete endoscopic examination of the esophagus, stomach and proximal duodenum was performed after an overnight fast, using a 9 mm endoscope (Olympus, Sweden). All subjects were offered topical anesthetics and intravenous midazolam. The position of the diaphragmatic cruz was identified having the patient sniff and the position of the gastroesophageal junction by the proximal margins of the longitudinal gastric rugal folds. Hiatal hernia was identified when the distance between the crural impressions to the GEJ was 2 cm or more. The SCJ was identified by the demarcation between squamous epithelium and columnar mucosa in the area of the GEJ. Erosive esophagitis was graded according to the Los Angeles classification (75). A columnar lined esophagus (CLE) was suspected when any part of the SCJ extended above the distal gastric rugal folds. The SCJ was carefully and systematically examined during gentle insufflation and exsufflation allowing for a dynamic evaluation. The localization and the geometry of the SCJ was assessed in reference with the GEJ and was graded according to the ZAP classification (166). The distal esophagus with the GEJ and the SCJ was carefully observed and video documented to assist consensus in inter observer agreement on the appearance of the GEJ, esophagitis, CLE, ZAP grade and hernia. The documentation could be used in the re-evaluation in uncertain cases.

Wireless 48 h pH- monitoring

The same technique and application and wireless pH system (Bravo, Medtronic, Shoreview, MN, USA), was used in all studies. All patients were instructed to cease PPIs 10 days before and H2 antagonists 7 days before the examination. Antacids were allowed until 24 h before the pH test. The pH capsule was calibrated in buffer solutions of pH 1.0 and pH 7.0 and activated by a magnetic switch before placement. The unique identification code sent by the transmitter each capsule and registered by the receiver was used to separate the recordings. The delivery system was introduced trans orally with the endoscope placed in the esophagus. Insufflated air in the stomach was evacuated and the pH-probe withdrawn from the stomach until the pH electrode on the tip of the capsule was placed immediately the SCJ. The distance between the incissors and the SCJ was measure with the delivery catheter, which was subsequently withdrawn so that the pH electrode was positioned no less than 5 mm just above the SCJ. Under direct guidance with the endoscope the pH probe was placed with the well in contact with the mucosa and suction applied for 45 s with the external vacuum pump to capture the mucosa into the cavity of the well securing it with the stainless pin. The delivery system was removed and the position of the capsule visually ensured neither placed to high nor dipping with the electrode below the SCJ. A second catheter system was placed similarly at 6 cm above the SCJ, which is suggested to approximate the conventional position for the catheter-based pH electrode 5 cm above the LES identified via manometry(167). Patients with the capsule inadvertently positioned with the electrode more than 5 mm above the SCJ were excluded.

The pH recording was initiated immediately after the placement of the wireless capsule. All participating individuals were instructed to keep a diary and to record symptoms, hours of meals, time in supine and upright position. The participants were encouraged to continue daily normal routines at work and at home and were instructed to keep the receiver attached around the waist with a belt. In case of necessity to remove the belt in daily hygienic routines, such as showering, they were instructed to keep the receiver as close as possible. No restrictions in amount of food intake were given except for avoid acidic containing products, alcohol and to keep tobacco use at a minimum.

Analysis of pH tracings

Paper I-V

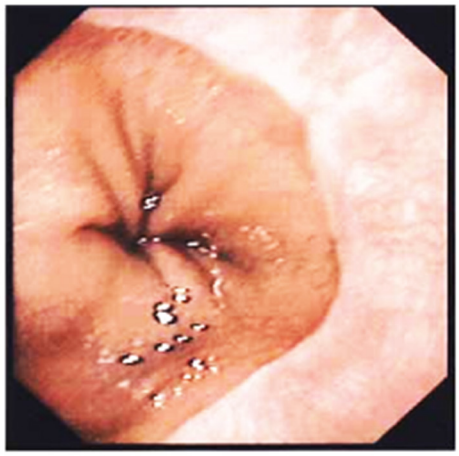
The monitored time was defined as the time from activation of the capsule receiver system immediately after the endoscopic examination until the receiver was switched of by the endoscopy nurse or stopped by it self automatically after 48

hours. The Bravo™ pH system sample frequency is every 6 seconds. When returned the data from the receiver was downloaded to a computer and analysed using Polygram™ NET (Medtronic, MN, USA). In the event of premature detachment of the pH capsule the event was detected in the pH tracing and manually corrected before analysis. Signal interruption data was defined as periods when the data signal was interrupted without any recording of pH data. The period of pH monitoring encompassed 48 hours. The 24-hour period with the highest esophageal acid exposure was defined as the worst day occurring either day 1 or day 2. The software programme calculated all characteristics of esophageal acid exposure except for in study III, where reflux episodes and the nadir pH were measured manually on the computerized pH tracings.

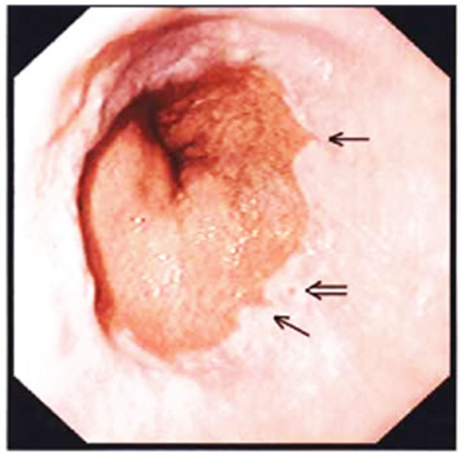
Symptom evaluation

Study II

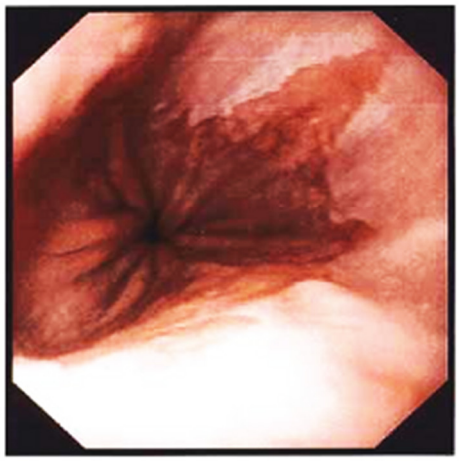
Symptom evaluation with the symptom indexes SI (sensitivity index) SSI and SAP (Symptom association probability) were used to study the presence and frequency of symptoms during the 48 h-hour pH-monitoring period. The standard software programme (Polygram™) for analysis of pH data automatically calculated the symptom indexes. The SI is defined as the percentage of reflux episodes associated symptoms and the SSI as the percentage of symptoms associated with reflux events. The SAP is a statistical method to determine the relationship between symptoms and reflux episodes(157). In study II the analysis of SI and SSI did not generate significance and therefore were not used in the final analysis. SAP $\geq$ 95% is considered a positive symptom-reflux association (153). In our study a SAP $\geq$ 95% for either heartburn or regurgitation was defined a positive test. As more patients had the symptom of heartburn compared with regurgitation, the analysis of SAP for heartburn was selected for the further analysis



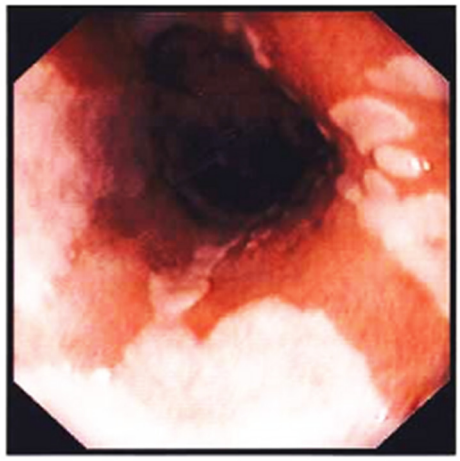
ZAP grade 0.
The Z-line is sharp and circular. The Z-line may be wave-like, due to the mucosal folds, but no tongues or islands of columnar epithelium is allowed in the esophagus.



ZAP grade I.
The Z-line is irregular and shows tongue-like protrusions (filled arrows) and an island (open arrow) of columnar epithelium.



ZAP grade II.
A distinct, obvious tongue of columnar epithelium < 3 cm is seen. A minimum requirement is that the base of the tongue is clearly shorter than the height.



ZAP grade III.
Distinct tongues of columnar epithelium > 3 cm, or a cephaled displacement of the Z-line > 3 cm.

Figure 3 pictures the different ZAP grades in the ZAP classification. Reprinted by permission from B.Wallner.

The ZAP classification

Study IV and V

The appearance of the SCJ was systematically graded according to the Z-line appearance (ZAP) classification introduced by Wallner et al (166).

ZAP grade 0 was defined as a sharp, distinct and circular SCJ, which could be wave like due to the mucosal folds but disappeared with insufflation with air. Irregularities were not allowed. ZAP grade I was defined by a circular SCJ with one or more small irregularities, no longer than 5 mm that remained visible during insufflation of air. Distinct tongues of columnar mucosa extending into the esophagus were not allowed. ZAP grade II was defined by a SCJ with a base that coincided with position of the GEJ but with the presence of tongues of columnar epithelium shorter than 3 cm. In the presence of distinct, obvious tongues of columnar epithelium longer than 3 cm or a cephalic displacement of the entire SCJ more than 3 cm, the SCJ was classified as ZAP grade III.

Statistical analysis

Papers I-V

As the data was not normally distributed, comparisons were made using nonparametric tests and the results were reported as medians and interquartile ranges unless otherwise stated. Analysis of continuous data was made using the Wilcoxon´s signed ranks test for intra individual data and the Mann Whitney U test were used to compare continuous data between individual groups. For proportions, independent categorical data were analysed using the chi square test or Fischer´s exact test and dependent data was analysed using McNemar sign test. For comparisons between more than two groups, we used the Kruskal-Wallis test for continuous data and McNemar´s sign test for categorical data.

Paper I and II

Analysis of continuous data was made using the Wilcoxon´s signed ranks test for intra individual data. Receiver operating characteristics (ROC) curves were used to graphically describe the trade off between sensitivity and specificity for different parameters of esophageal acid exposure. The ROC curve plots sensitivity versus (1-specificity) where each pair of values is generated by the defined cut-off value(168). By changing the thresholds of either sensitivity or specificity a cut off level may be obtained that may be clinically relevant. A predefined threshold with specificity at 90% was chosen to compare the sensitivity between the two examined anatomical positions in the esophagus. The ability of a test to correctly classify normal from abnormal can be described in the terms of “diagnostic

accuracy"(169). The diagnostic accuracy is directly related to the AUC where a value of 1.0 represents perfect discrimination whereas 0.5 represents a complete absence of discrimination. AUC values between 0.7-0.8 generally represent moderate accuracy and a value above 0.9 represents highly accurate tests. The diagnostic accuracy was of the wireless pH test was analysed by calculating the AUC and the results were presented with 95% confidence limits. The cutoff value for 90 % specificity has been obtained from observations in a non-diseased healthy population and is subject to random variation. The sensitivity values depend on the randomness in both the diseased and healthy population. The confidence levels for these values were calculated using Linnet´s non-parametric approach. Comparison of proportions between groups was performed using McNemar´s test. In paper I the SPSS 12.0 was used and in paper II the SPSS 20.0 was used.

Paper IV and V

The distribution of the data was tested using the Kolmogorov-Smirnov test of normality. In paper V Bonferroni correction was made for comparison of more than two groups. In paper IV the variables that reached a p-value of 0.1 or less in the univariate analyses were entered in a binary logistic regression analysis in order to assess their relative importance for the presence of IM at the GEJ. This analysis was made using a backward stepwise selection with removal testing based on the probability of the Wald statistic. The SPSS 22.0 was used in paper IV and SPSS 21.0 in paper V.

Results

Paper I

A total of 70 patients and a control group containing 55 healthy subjects entered the study. Three patients were excluded due to circumferential segment of columnar mucosa and one patient because of a peptic stricture. Another 4 patients were excluded due to technical problems with the wireless pH system. In the control group 6 patients were excluded due to simultaneous pH recordings couldn´t be obtained due to technical problems with the distal pH capsule. In total 62 patients and 49 healthy volunteers with successful dual pH recording were analysed in the study. The demographic data of patients and control subjects are shown in table 1. The age in the control group was evenly distributed within each 5-year interval. No difference in gender distribution was seen but the patient group was significantly older and had a higher body mass index (BMI) compared with the healthy volunteers.

Table 1. Demographic data of asymptomatic controls and patients with typical refluxsymptom

	Control n=49	All Patients n=62	*p*
Age, years	42(32-50)	48(40-59)	0.031
Gender, m/f	25/24	39/23	0.25
BMI, kg/m3	24(22-26)	28(25-30)	<0.0001

Median (IQR)

Thirty patients had no endoscopic evidence of mucosal injury and 32 patients (52%) had esophagitis. Fifteen patients had grade A esophagitis and 17 patients had grade B esophagitis according to the LAC. There were no patients with severe esophagitis grade C or D. The patients were asked to report presence or recurrence of reflux symptoms after discontinuing acid suppressive medication to confirm and document presence of acid reflux related symptoms. Fifty-eight out of 62 patients (94%) reported recurrence of symptoms at the time of the pH test. In the intention to ensure a representative GERD population the patients were asked to report any recurrence of reflux symptoms after discontinuing acid suppressive therapy prior to the study. Relapse was reported by 58 of the 62 patients (94%), whereof one patient from the esophagitis group and three from the group without mucosal injuries

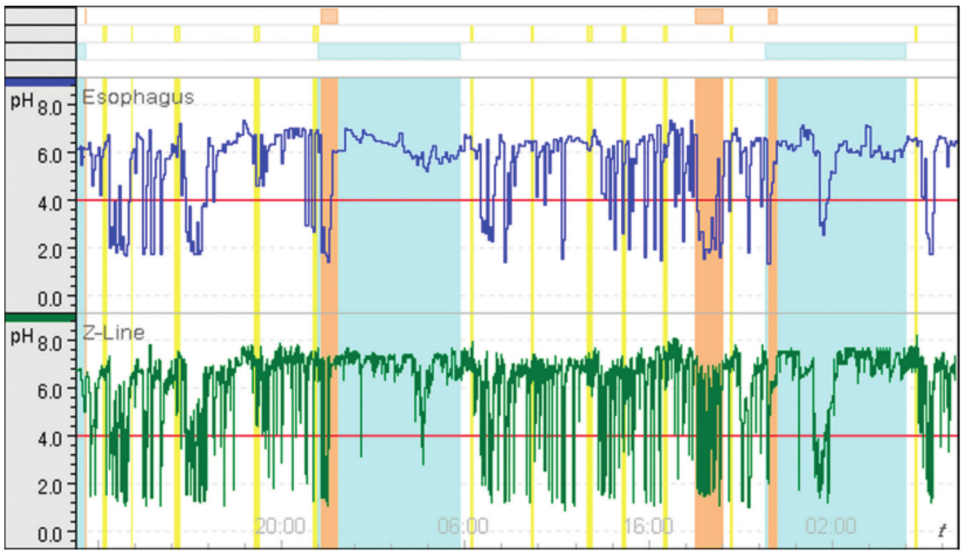


Fig 4. pH tracing showing simultaneous 48-hour esophageal pH monitoring in a healthy subject using the wireless pH system. One pH electrode positioned 6 cm above (Esophagus) and one pH electrode positioned immediately above the squamocolumnar junction (SCJ). Coloured fields represent meal periods (yellow) and supine posture (blue).

In fig 4 the simultaneous pH recording of the 48-h at the SCJ and at the 6 cm esophageal level in a patient with GERD is shown. Table 2 shows the characteristics of esophageal acid exposure simultaneously recorded immediately above and 6 cm above the SCJ in patients and in healthy controls. The degree of esophageal acid exposure recorded by the distal capsule was significantly higher compared to that detected by the proximal electrode in control subjects ($P < 0.0001$) as well as in symptomatic patients ($P < 0.0001$). Within the control group and the patient group there were no significant differences in the degree of acid exposure between day 1 and day 2. The median 48-h total % time with esophageal pH<4 recorded immediately above the SCJ was 10.0% (8.5–13.9) in patients with esophagitis and 6.6% (4.4–12.0) in patients with no mucosal injury ($P = 0.007$). The corresponding percentage of time with pH < 4 for patients with and without esophagitis measured 6 cm above the SCJ was 6.8% (4.1–9.3) and 3.4% (2.2–5.7), respectively ($P < 0.003$).

To select a parameter of esophageal acid exposure for the subsequent analysis of sensitivity and specificity, different parameters were analysed using the AUC (Table 3)

Table 2. Parameters of esophageal acid exposure obtained by simultaneous pH monitoring immediately above the squamocolumnar junction (SCJ) and 6 cm above the SCJ in healthy controls and patients with typical reflux symptoms

	Controls		All patients	
	SCJ	Esophagus	SCJ	Esophagus
Monitored time h:min	46:33	46:28	45:50	45:52
48-h total % time pH<4	1.5(1.0-4.0)	0.0(0.4-2.0)	9.5(6.5-13.4)	5.4(2.6-8.3)
Day 1 total % time pH<4	1.6(0.7-3.5)	0.6(0.2-1.9)	9.2(6.2-13.1)	5.0(2.5-8.7)
Day 2 total % time pH<4	1.5(0.8-3.0)	0.9(0.3-1.8)	9.7(6.6-11.6)	5.0(2.5-8.4)
Worst day % time pH<4	2.3(1.2-5.4)	1.4(0.5-3.1)	10.3(7.1-15.5)	6.2(3.2-9.3)

Median (IQR)

Table 3. The area under the ROC curves for different parameters of esophageal acid exposure for 48-h wireless pH monitoring immediately above the squamocolumnar junction (SCJ) and 6 cm above the SCJ (Esophagus)

	All Patients n=62		No Esophagitis n=30		Esophagitis n=32	
	SCJ	Esophagus	SCJ	Esophagus	SCJ	Esophagus
48-h total % time pH<4	0.917(0.86-0.98)	0.901(0.85-0.96)	0.884(0.81-0.96)	0.854(0.77-0.93)	0.948(0.90-1.00)	0.945(0.90-0.99)
Day 1 total % time pH<4	0.903(0.84-0.96)	0.885(0.83-0.94)	0.865(0.79-0.94)	0.830(0.74-0.92)	0.938(0.88-1.00)	0.937(0.88-0.99)
Day 2 total % time pH<4	0.899(0.83-0.96)	0.873(0.81-0.94)	0.864(0.78-0.94)	0.824(0.74-0.92)	0.933(0.87-0.99)	0.919(0.86-0.98)
Worst day total % time pH<4	0.894(0.83-0.96)	0.877(0.81-0.94)	0.860(0.78-0.94)	0.824(0.74-0.91)	0.927(0.87-0.99)	0.926(0.87-0.98)

AUC (95% Confidence Limits)

Although not statistically tested, the total percentage of time that esophageal pH was less than 4 for the entire 48-h study period had the highest AUC values irrespective of the level of pH recording, which indicates that this was the parameter that best distinguished patients from controls. ROC curves for pH monitoring immediately above the SCJ and at the standard electrode position (Figs. 5A–C) are plotted for all patient groups.

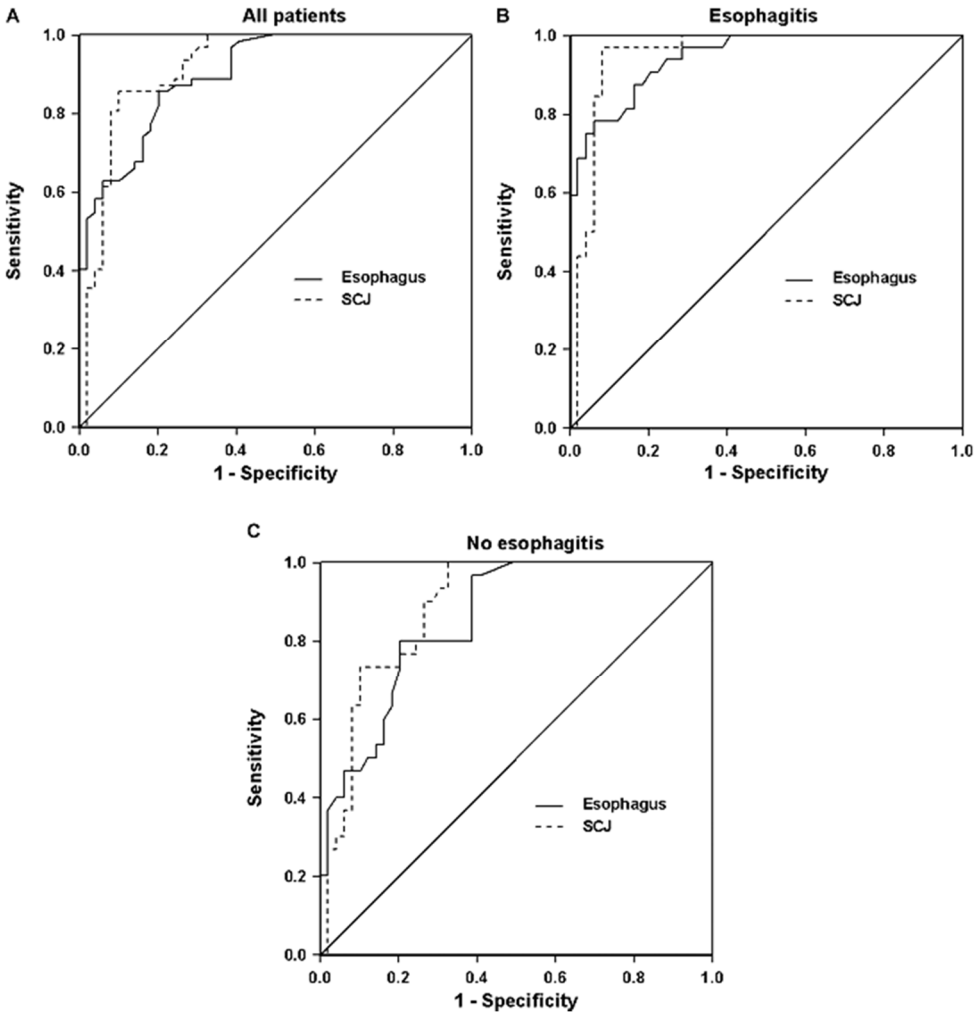


Figure 5. (*A*–*C*) Receiver operating characteristics (ROC) curves calculated for the 48-h total % time that pH was below 4 for all patients with reflux symptoms (*A*) and the subgroups of patients with (*B*) and without esophagitis (*C*). Each graph demonstrates the ROC curve generated for pH recording immediately above the squamocolumnar junction (SCJ) and 6 cm above the SCJ (Esophagus).

ROC analysis separately for day 1, day 2, or using the day with the highest acid exposure (worst day) consistently generated lower AUC values suggesting a lower diagnostic accuracy. Therefore, the total percent time that pH was less than 4 for the entire study period was the parameter chosen for the subsequent analysis of sensitivity and specificity at the two levels of pH monitoring. Esophageal pH monitoring performed immediately above the SCJ generated higher AUC values for all patients as well as for the subgroups of patients with and without esophagitis, indicating a higher diagnostic accuracy compared with pH monitoring using standard electrode placement. Sensitivity and specificity of a diagnostic test are inversely related and can be calculated within the ROC curve. In general the AUC values generated by the ROC curve reflect the discriminatory power of a diagnostic test. However, AUC values reflect the performance of the test based on all intervals of the curve including areas under the curve that are clinically irrelevant. Therefore the shape of the ROC curve and the performance of the test in clinically relevant intervals are most important. In order to compare possible differences in sensitivity of the pH test at the two levels, a predefined level of specificity was chosen. To be useful in the clinical management of patients with reflux symptoms, we believe that a test specificity of 90% is necessary. Within the ROC curve, this level of specificity generated a cutoff value for the 48-h % time that pH was <4 of 5% at the level immediately above the SCJ and 3.6% at the conventional level for wireless pH recording. Using these cutoff levels, the sensitivity of wireless pH recording at the two levels was calculated for all patient groups. Compared with standard electrode placement, pH recording immediately above the SCJ increased the sensitivity of esophageal pH monitoring from 63% to 86% in all patients, from 47% to 73% in patients with no mucosal injury and from 78% to 97% in patients with esophagitis. pH recording performed in the most distal esophagus significantly increased the number of patients correctly classified with GERD (Table 4).

Table 4. Sensitivity of 48-h Wireless pH Monitoring in GERD Patients Measured Immediately Above the Squamocolumnar Junction (SCJ) and at the Standard pH Electrode Position 6 cm Above the SCJ (Esophagus) at a Predefined Test Specificity of 90%

	SCJ		Esophagus		
	Sensitivity (95% CI)	Proportion of Patients Correctly Classified With GERD	Sensitivity (95% CI)	Proportion of Patients Correctly Classified With GERD	*P*
All patients (N = 62)	0.85 (0.71–1.0)	53/62	0.63 (0.41–0.85)	39/62	0.00018
No esophagitis (N = 30)	0.73 (0.51–0.96)	22/30	0.47 (0.20–0.73)	14/30	0.0047
Esophagitis (N = 32)	0.97 (0.90–1.0)	31/32	0.78 (0.58–0.99)	25/32	0.014

Paper II

A total of 128 patients with symptoms suggestive of GERD underwent upper endoscopy followed by a dual ambulatory 48-hour pH monitoring. Seventy of the patients and 55 of the asymptomatic volunteers had participated in a the previous study I. Forty subjects were excluded due to atypical symptoms or insufficient

response to proton inhibitor therapy and another six patients due to contraindications to the pH capsule system or due to technical problems as previously described in study I. The control group consisted after the exclusion as described in study I of 49 asymptomatic volunteers. Demographical and clinical data of patients and asymptomatic volunteers are shown in Table 5. There was no difference in gender distribution but the patients were significantly older and had a higher body mass index (BMI) than asymptomatic controls. The symptomatic patients were divided in two groups based on the presence or absence of erosive esophagitis. Thirty-five of the 82 (42.7%) patients had no mucosal injury and 47 patients had erosive esophagitis. Of these, 26 (55.3%) patients had grade A and 21 (44.7%) patients had grade B esophagitis according to the Los Angeles classification.

Table 5. Demographic data of asymptomatic controls and patients with typical refluxsymptom

	Control n=49	All Patients n=82	*p*
Age, years	42(32-50) [22-65]	49(40-59) [20-73]	0.013
Gender, m/f	25/24	51/31	0.210
BMI, kg/m3	24(22-26.5)	28(24.9-30.2)	<0.0001
Hiatal Hernia	3/49 (6.1%)	62/72 (75.6)	<0.0001
Erosive esophagitis	0/49 (0%)	47/82 (57.3%)	<0.0001

Median (IQR)

Esophageal pH monitoring

The degree of esophageal acid exposure recorded simultaneously in the most distal esophagus and 6 cm above the SCJ in patients and asymptomatic controls are shown in Table 6.

Table 6. Degree of esophageal acid exposure obtained by simul- taneous pH recording immediately above the SCJ (SCJ) and 6 cm above the SCJ (Esophagus) in healthy asymptomatic volunteers and in patients with typical reflux symptoms.

	SCJ	Esophagus	*p*
Asymptomatic volunteers	1.6 (0.3–10.6)	0.9 (0.0–4.4)	<0.001
All patients	9.8 (6.8-14.4)	5.0(2.8-8.8)	<0.001
No mucosal injury	6.9(4.5-12.8)	3.5(2.2-5.6)	<0.001
Erosive esophagitis	10.7(8.5-14.1)	6.8(4.1-9.5)	<0.001

The percent time with pH < 4.0 was significantly higher in the most distal esophagus compared with that observed 6 cm above the SCJ in asymptomatic controls as well as in patients with reflux symptoms.

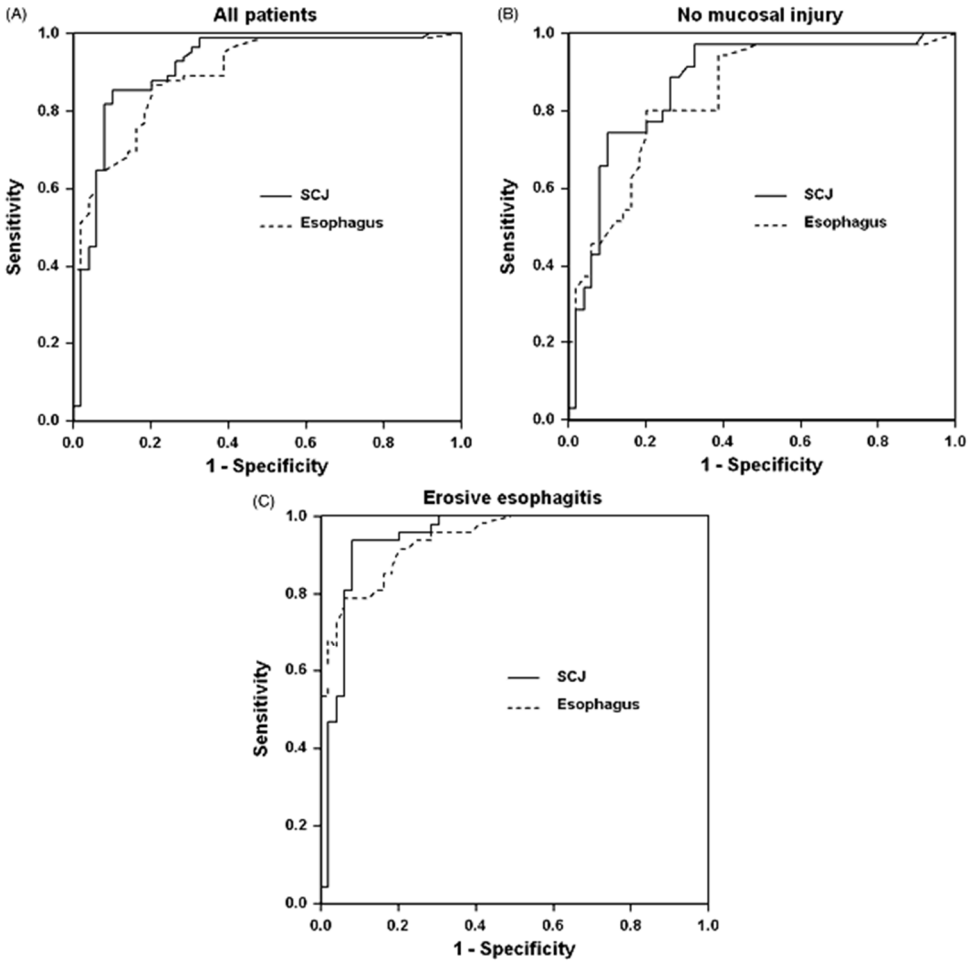


Figure 6. Receiver operating characteristics (ROC) curves calculated for the 48-h total time that esophageal pH was below generated by pH recording immediately above the squamocolumnar junction (SCJ) and the standard electrode position, 6 cm above the SCJ (Esophagus) for all patients with reflux symptoms (A), for patients with no mucosal injury (B) and for patients with erosive esophagitis (C).

ROC curves were plotted for the two levels of pH monitoring (Figure 6A, B and C). In all patients, the AUC value for pH monitoring just above the SCJ was 0.91 (95% confidence intervals 0.84–0.97), which was numerically greater than that obtained for pH monitoring 6 cm above the SCJ (0.89, 95% CI 0.85–0.95). For the subgroups of patients with no mucosal injury and erosive esophagitis, the corresponding AUC values were 0.87 (95% CI 0.79–0.95) versus 0.83 (95% CI 0.75–0.92) and 0.95 (95% CI 0.89–0.99) versus 0.94 (95% CI 0.89–0.98), respectively. Within the ROC curve, the predefined specificity of 90% generated

the cut off value 5.7 % for the percent of time that esophageal pH was less than 4 for pH monitoring performed immediately above the SCJ and 3.6% for the standard level. Using these thresholds for abnormal esophageal acid exposure, the sensitivity of capsule based pH monitoring at the two levels was calculated. Compared with standard electrode placement, pH monitoring in the most distal esophagus significantly increased the sensitivity of the test (Table 7). In all patients with GERD the sensitivity increased from 64.6% to 81.7% and in the subgroups of patients with and without erosive esophagitis the sensitivity increased from 78.7% to 93.6% and 45.7% to 65.7%, respectively.

Table 7. Sensitivity of pH monitoring performed 6 cm above the SCJ (Esophagus) and immediately above the SCJ (SCJ) and the sensitivity of the combination of abnormal acid exposure and/or positive SAP in patients with typical reflux symptom of heartburn. Sensitivity of pH monitoring was calculated from the ROC-curve with a predefined specificity of 90%.

	SCJ	Esophagus	*p*-Value
All patients *n=82*			
Abnormal acid exposure	81.7%	64.6%	0.001
Abnormal acid exposure and/or positive SAP	93.6%	80.5%	<0.001
No mucosal injury *n=35*			
Abnormal acid exposure	65.7%	45.7%	0.039
Abnormal acid exposure and/or positive SAP	94.3%	74.3%	0.016
Erosive Esophagitis *n=47*			
Abnormal acid exposure	93.6%	78.7%	0.016
Abnormal acid exposure and/or positive SAP	97.9%	85.1%	0.031

SAP=Symptom Association Probablility

Symptom association analysis

The results of the symptom association analyses at the two levels of pH recording are shown in Table 8.

Table 8. Proportion of positive symptom association analyses obtained by simultaneous pH recording immediately above the SCJ (SCJ) and 6 cm above the esophagus (Esophagus) in patients with heartburn.

	SCJ	Esophagus	*p*
% Positive SAP	62/82 (75.6%)	51/82(62.2%)	0.001
% Positive SI	51/82 (62.2%)	38/82 (46.3%)	0.002
% Positive SSI	34/82 (41.5%)	37/82 (45.1%)	0.453

SAP=Symptom Association Probability, SI=Symptom Index, SSI=Symptom Sensitivity Index

As all 82 patients experienced symptoms of heartburn but only 47 patients reported symptoms of regurgitation, the symptom analysis was further directed on the symptom of heartburn. The diagnostic yield of SAP and SI analyses was significantly higher greater for pH monitoring in the most distal esophagus compared with that observed at the conventional level but no such difference was observed for the SSI. The highest proportion of positive symptom association

analyses was generated by the SAP, indicating that this test is superior in detecting a temporal association between reflux events and symptoms. The diagnostic performance of the pH test improved significantly when a combination of abnormal acid exposure and/or a positive SAP was used as a marker for a positive pH test (Table 7). In all patients with reflux disease as well as in the subgroups with and without erosive esophagitis, the proportion positive pH tests was significantly higher for pH monitoring performed in the most distal esophagus. The results of improved diagnostic accuracy of pH monitoring performed immediately above the SCJ from paper I was confirmed in paper II in a larger similar study population. Using a predefined specificity of 90%, the sensitivity of esophageal pH monitoring and the diagnostic yield of Symptom Association Probability (SAP) were significantly higher for pH monitoring performed at the distal compared with the conventional level (82% vs. 65%, $p<0.001$ and 76% vs. 62%, $p<0.001$, respectively). The greatest improvement was observed in patients with non-erosive disease. In this group, the sensitivity increased from 46% at the standard level to 66 % immediately above the SCJ, and with the combination of a positive SAP as a marker for a positive pH test, the diagnostic yield further increased to 94%.

Paper III

A total of 82 patients with typical symptoms of reflux and 55 healthy volunteers entered the study and participants with at least 36 h successful distal pH monitoring participated in the analysis. Sixty-two patients had participated in earlier studies for evaluation of the wireless pH system and another 13 were included meeting the same criteria. 5 of the healthy volunteers were excluded du to technical problems. Thus the analysis included pH data of in total 75 patients and 50 asymptomatic volunteers. Thirty-two of the 75 patients (42.7%) had no evidence of esophageal mucosal injury on endoscopy. Of the 43 patients with erosive disease, 22 (51.2%) were found to have grade A and 21 grade B esophagitis according to the Los Angeles classification [16]. None of the patients had severe esophagitis (Los Angeles grade C or D). Demographic data of the patients and control subjects are shown in Table 9.

Table 9. Demographic data of asymptomatic volunteers and patients with and without erosive disease

	Asymptomatic volunteers	Non-erosive reflux disease	Erosive reflux disease	p-value
N	50	32	43	
Gender (m/f)	26/24	16/16	31/11	0.042
Age	42 (32-50)	42 (35-51)	53 (42-62)	0.001
BMI	24.0 (22.0-26.3)	26.8 (23.9-28.9)	28.3 (25.2-30.6)	<0.001

Patients with erosive esophagitis were predominantly male and older than asymptomatic volunteers and the patients with non-erosive disease. A hiatal hernia was found in 15 of the 32 (47%) patients with no endoscopic evidence of mucosal injury and in 40 of the 43 (93%) patients with erosive esophagitis. Table 10 shows the characteristics of pre- and post- prandial esophageal acid exposure recorded immediately above the SCJ in patients and in healthy controls.

Table 10. Characteristics of esophageal acid exposure immediately above the squamocolumnar junction

	Preprandial periods	**Postprandial periods**	***p***
Asymptomatic volunteers n=49			
Monitored time (h)	18.7 (15.0-20.7)	9.0 (8.6-10.1)	<0.001
% time pH < 4.0	2.5 (1.10-5.15)	2.2 (1.15-4.62)	0.165
# reflux episodes/hour	1.7 (1.01-3.19)	2.5 (1.42-3.92)	0.004
Duration of reflux events (min)	0.4 (0.3-1.1)	0.3 (0.1-0.8)	<0.001
Duration longest reflux event (min)	4.0 (2.0-7.5)	3.0 (1.0-5.0)	0.086
Non-erosive reflux disease n = 32			
Monitored time (h)	16.0 (14.0-19.3)	10.4 (9.0-11.9)	<0.001
% time pH < 4.0	7.6 (4.7-10.6)	10.9 (6.5-18.9)	0.003
# reflux episodes/hour	4.1 (3.1-6.3)	6.3 (4.8-8.4)	<0.001
Duration of reflux events (min)	0.5 (0.1-1.4)	0.4 (0.1-1.2)	<0.001
Duration longest reflux event (min)	8.0 (5.0-13.5)	8.0 (5.2-13.0)	0.964
Erosive reflux disease n = 43			
Monitored time (h)	17.6 (13.8-20.2)	9.9 (8.6-11.8)	<0.001
% time pH < 4.0	9.7 (6.6-13.1)	16.8 (13.3-22.5)	<0.001
# reflux episodes/hour	5.0 (3.2-6.1)	7.9 (6.2-9.7)	<0.001
Duration of reflux events (min)	0.6 (0.2-1.7)	0.5 (0.1-1.5)	<0.001
Duration longest reflux event (min)	10.0 (7.0-15.0)	11.0 (7.0-20.0)	0.394

Values reported as medians and interquartile ranges

There was a progressive increase in the degree of esophageal acid exposure from control subjects to patients with no mucosal injury and those with erosive esophagitis. The increasingly higher degree of acid exposure was observed in the preprandial as well as the postprandial periods. The percentage of time with pH below 4.0 was significantly higher in the postprandial compared with the preprandial state in patients with typical symptoms of GERD but no such difference was observed in control subjects. The higher degree of esophageal acid exposure in the most distal esophagus during the postprandial periods was not due to prolonged reflux events as suggested by the acid pocket theory, but rather to

numerous short reflux events. This was illustrated by the median duration of reflux events, which was significantly shorter in the postprandial states, and the median duration of the longest reflux episode, which was similar in the two states. All reflux episodes were stratified into different groups based on their duration. Figure 7A-C shows the distribution of reflux events with varying durations in asymptomatic volunteers and symptomatic patients.

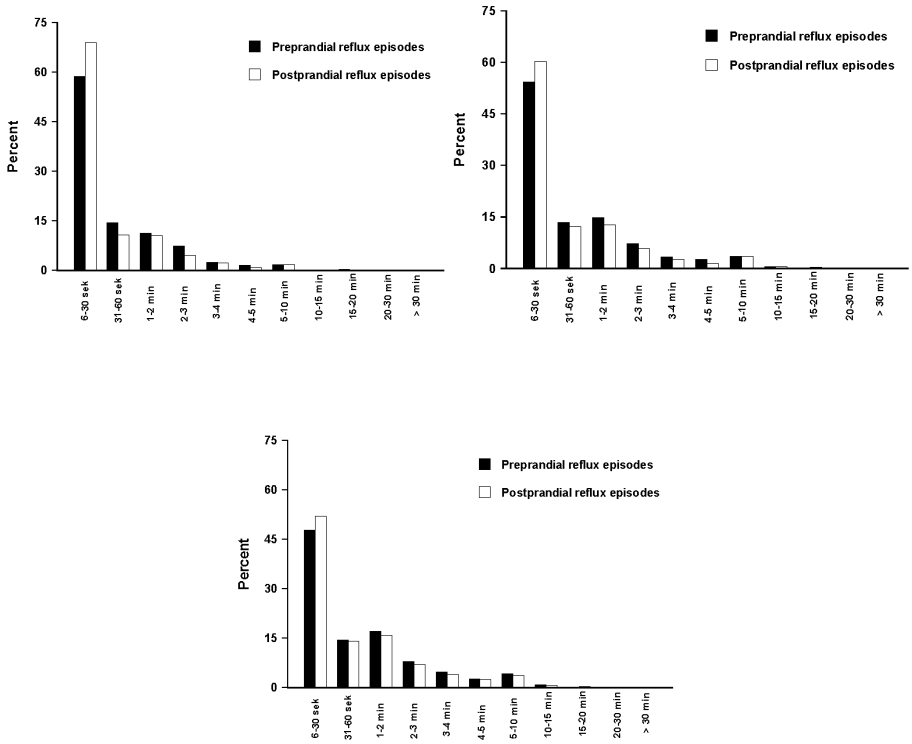


Figure 7 A-C. Distribution of pre- and postprandial refluxevents of varying durations in asymptomatic (A) and asymptomatic patients with no mucosal injury (B) and ersoive esophagitis (C)

In all study groups, the majority of preprandial and postprandial reflux events were of a short duration (6 – 60 s). The proportion of such short reflux events was significantly higher in the postprandial compared with the preprandial periods in control subjects ($p < 0.001$) and symptomatic patients ($p < 0.02$). Long reflux events were rare observations. In the postprandial states, reflux events with duration longer than 5 min were observed with a median frequency of 0.37 per hour in patients with esophagitis and 0.35 per hour in patients without mucosal injury.

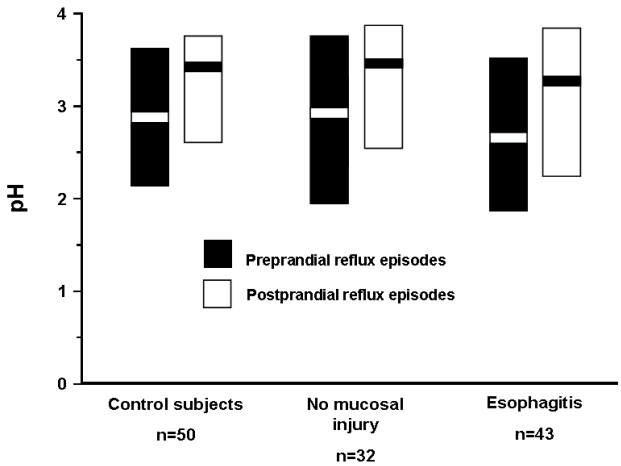


Figure 8. Median minimum pH of pre- and postprandial reflux events in asymptomatic volunteers and in symptomatic patients with and without esophagitis.

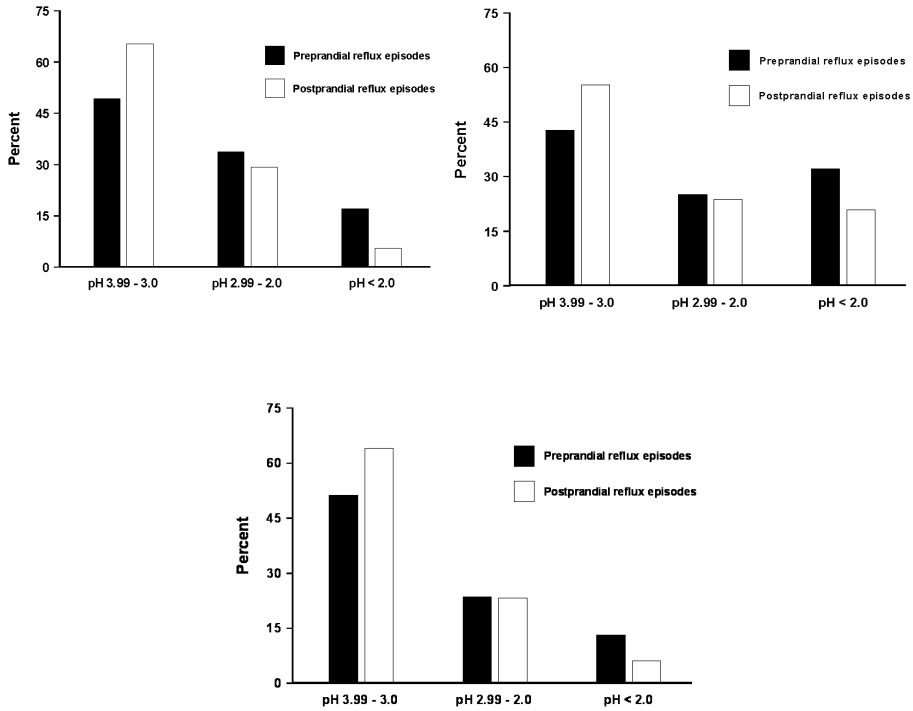


Figure 9 A-C. Proportions of pre- and postprandial reflux events with minimum pH less than 2, 3 or 4, recorded immediately above the SCJ during pH monitoring in asymptomatic volunteers (A) and in symptomatic patients without (B) and with esophagitis (C).

In all study groups, the acidity of postprandial reflux events was significantly lower than that observed for preprandial reflux events. This was demonstrated by a significantly higher minimum pH of reflux events in the postprandial compared with the preprandial period (Figure 8). The differences in acidity of the reflux events was further demonstrated by a significantly lower proportion of reflux events with a nadir pH below 2 or 3 in the postprandial states (Figure 9A–C).

Paper IV

The study included 149 patients with symptoms suggestive of GERD and 50 healthy volunteers. Fifteen of the asymptomatic volunteers and 35 of the symptomatic patients were excluded from the analysis as they had irregular SCJs of metaplastic columnar mucosa extending 5 mm or more above the base of the SCJ and the aim of the study was to evaluate IM in individuals with endoscopically normal appearing GEJs. Consequently, the analysis included 35 asymptomatic volunteers and 114 patients with symptoms suggestive of GERD. BMI was significantly higher in symptomatic patients compared with the

asymptomatic volunteers but there was no difference in age or gender distribution between the groups (Table 11).

Table 11. Demographic data of healthy volunteers and symptomatic patients

	Asymptomatic volunteers	Symptomatic patients	*p*-value
Gender (male/female)	24:33	52:62	.063
Age	42 (33-50)	47 (37 - 57)	.089
BMI	24.0 (22.0-26.0)	25.9 (22.4 - 29.5)	.017
Tobaco %	0.0 (0/47)	7.0 (8/114)	.0105

Hiatal hernia, erosive esophagitis, ASA-treatment and a history of HP eradication therapy were significantly more prevalent in patients compared with asymptomatic volunteers (Table 12).

Table 12. Clinical characteristics of healthy volunteers and symptomatic patients

	Asymptomatic volunteers	Symptomatic patients	p-value
Hiatal hernia	4.3% (2/47)	63.2% (72/114)	< 0.001
Erosive esophagitis	0.0% (0/47)	30.7% (35/114)	< 0.001
ZAP grade I	46.8% (22/47)	86.8% (99/114)	< 0.001
IM gastric antrum	2.1% (1/47)	0.9% (1/114)	0.500
IM GEJ	2.1% (1/47)	26.3% (30/114)	0.296
HP infection	31.9% (15/47)	7.0% (8/114)	0.747
Previous HP eradication	8.5% (4/47)	8.8% (10/114)	0.035
HP infection or previous HP eradication	0.0% (0/47)	14.0% (16/114)	0.435
ASA	8.5% (4/47)	7.9% (9/114)	0.060

IM = intestinal metaplasia, GEJ= Gastroesophageal junction, ZAP=Z-line appearance, HP= *H. Pylori,* ASA= Acetylsalicylic acid

Esophagitis was found in 35/114 (30.7%) and IM at the GEJ was found in 45 of the 161 participants (28%). There was no significant difference in the prevalence of IM at the GEJ between asymptomatic volunteers and patients. The demographic data was similar in subjects with and without IM (Table13).

Table 13. Demographic characteristics of subjects with and without IM at the GE

	No IM *n*=116	IM *n*=45	*p*-value
Age	44 (35-56)	49 (37-47)	0.466
Gender (male/female)	55/61	21/24	1.000
BMI	25.0 (22.2-29.1)	24.9 (22.0-27.7)	0.281

IM = intestinal metaplasia, GEJ= Gastroesophageal junction, BMI=Body Mass Index

The clinical characteristics of subjects with and without IM at the GEJ are shown in Table 14.

Table 14. Clinical characteristics of subjects with and without IM at the GE

	No IM n=116	IM n=45	p-value
No tobacco use	95.7% (111/116)	93.3% (42/45)	0.687
Tobacco use	4.3% (5/116	6.5% (3/45)	
No hiatal hernia	51.7% (60/116)	60.0% (27/45)	0.382
Hiatal hernia	48.3% (56/116)	40.0% (18/45)	
No erosive esophagitis	76.7% (89/116)	82.2% (37/45)	0.527
Erosive esophagitis	16.8% (27/116)	17.8% (8/45)	
ZAP grade 0	31.0% (36/116)	8.9% (4/45)	0.004
ZAP grade I	69.0% (80/116)	91.1% (41/45)	
No IM gastric antrum	99.1% (115/116)	97.8% (44/45)	0.482
IM gastric antrum	0.9% (1/116)	2.2% (1/45)	
No HP infection	94.0% (109/116)	88.9% (40/45)	0.318
HP infection	6.0% (7/116)	11.1% (5/45)	
No HP infection and no previous HP infection	90.5% (105/116)	80.0% (36/45)	0.107
HP infection or previous HP eradication	9.5% (11/116)	20.0% (9/45)	
No ASA use	93.1% (108/116)	97.8% (44/45)	0.447
ASA use	6.9% (8/116)	2.2% (1/45)	

IM = intestinal metaplasia, GEJ= Gastroesophageal junction, ZAP=Z-line appearance, HP= *Helicobacter Pylori,* ASA= Acetylsalicylic acid

IM was significantly associated with an endoscopic appearance of the SCJ corresponding to ZAP grade I but there was no association with H Pylori infection. Table 15 demonstrates the characteristics of acid reflux in subjects with and without IM at the GEJ.

Table 15. Characteristics of acid reflux measured immediately above the SCJ in subjects with and without IM on biopsies of a normal appearing GEJ.

	No IM *n*=116	IM *n*=45	*p*-value
% Time pH <4, total	3.5 (1.3-9.1)	6.5 (3.4-9.4)	0.082
% Time pH <4, supine	0.5 (0.0-4.4)	1.7 (0.2-4.0)	0.346
% Time pH <4, upright	5.2 (1.8-11.4)	7.1 (4.2-13.2)	0.092
% Time pH <4, postprandial	7.2 (2.0-17.2)	8.9 (4.0-16.8)	0.419
Number of reflux episodes/h	2.0 (1.1-3.8)	3.0 (1.8-4.6)	0.015
Number of reflux episodes > 5 min/h	0.06 (0-0.2)	0.08 (0.04-0.22)	0.330
Duration longest reflux episode (min)	10.0 (4.0-20.0)	16.0 (7.0-25.5)	0.056
Abnormal acid exposure (%)	38.8 (45/116)	57.8 (26/45)	0.023

SCJ = squamocolumnar junction, IM = intestinal metaplasia, GEJ= Gastroesophageal junction

IM was significantly associated with increasing frequency of reflux episodes and with abnormal esophageal acid exposure measured immediately above the SCJ. The factors that reached a p-value of 0.1 or less in the univariate analyses were entered in a binomial logistic regression analysis to assess their independent importance for the presence of IM at the GEJ (Table 16).

Table 16. Binary logistic regression showing the relative risk for IM at an endoscopically normal appearing GEJ.

	Relative risk	95% CI for relative risk	p-value
% Time pH <4 total	0.72	0.57 – 0.91	0.005
% Time pH <4 upright	1.00	0.84 – 1.20	0.975
Number of reflux episodes/h	1.49	1.14 – 2.15	0.031
Duration longest reflux episode (min)	1.02	0.99 – 1.04	0.205
Abnormal acid exposure (%)	5.50	1.23 – 24.55	0.026
ZAP grade I	4.65	1.39 – 15.62	0.013

IM = intestinal metaplasia, GEJ= Gastroesophageal junction, ZAP=Z-line appearance.

IM at the GEJ was significantly associated with increasing number of reflux episodes per hour (1.5 (1.1-2.2), p = 0.031), abnormal acid exposure (5.5 (1.2-24.6), p = 0.026) in the most distal esophagus and with an endoscopic appearance of the SCJ corresponding to ZAP grade I (4.6 (1.4- 15.6), p=0.013). Further, there was a small but significant negative association between IM at the GEJ and increasing percentage time with pH below 4.0 measured immediately above the SCJ.

Paper V

The study analysis consisted of 149 patients with typical and atypical symptoms of reflux and 50 asymptomatic patients with successful esophageal distal pH monitoring. The median age and BMI of the patients were significantly higher than that of the asymptomatic subjects but there was no significant difference in gender distribution. Table 17 shows the demographical characteristics of asymptomatic volunteers and symptomatic patients.

Table 17. Demographic data of asymptomatic volunteers and patients

	Asymptomatic subjects n=50	Symptomatic patients n=149	p-value
Age, years	42 (32-50)	49 (37.5-58.5)	0.025
Male/female	26/24	73/76	0.713
BMI	24.0 (22-26.2)	25.9 (23-29.4)	0.006

BMI = Body mass index. Values reported as medians and interquartile ranges

Ninety-one of the 149 (61%) participants had no endoscopic evidence of mucosal injury and 58 (39%) subjects had erosive esophagitis. Of the individuals with erosive disease, 32 (55.2%) had grade A and 26 (44.8%) had grade B esophagitis (LA). None of the participants had severe esophagitis (Los Angeles grade C or D). The distribution of ZAP grades in asymptomatic volunteers and patients is shown in Table 18.

Table 18. Distribution of ZAP grades in asymptomatic volunteers and patients

	Asymptomatic subjects n=50	All patients n=149	Patients atypical symptoms n=55	Patients typical symptoms n=94
ZAP grade 0	25 (50.0%)*	15 (10.1%)	12 (21.8%)	3 (3.2%)
ZAP grade I	22 (44.0%)*	99 (66.4%)	39 (70.9%)	60 (63.8%)
ZAP grade II	3 (6.0%)*	35 (23.5%)	4 (7.3%)	31 (33.0%)
ZAP grade III	0 (0%)	0 (0%)	0 (0%)	0 (0%)

ZAP = Z-line appearance. * $p < 0.005$ vs. all patients

The endoscopic finding of a well demarcated and uniform SCJ without irregularities was significantly more prevalent in asymptomatic volunteers compared with the patient group. Correspondingly, irregular SCJs were significantly more frequent and more pronounced in patients compared with asymptomatic subjects. As the manufacturer of the pH monitoring system considered the presence of segments of esophageal columnar mucosa of 3 cm or longer to be an contraindication for capsule-based pH monitoring, none of the participants had a SCJ corresponding to ZAP grade III. There was a significant association between the endoscopic appearance of the SCJ and the presence of erosive esophagitis. The prevalence of erosive esophagitis increased from 0% (0/42) in patients with ZAP grade 0 to 28.9% (25/ 121) and 60.5% (23/38) in subjects with ZAP grade I and II, respectively ($p < .001$). Table 19 shows the characteristics of esophageal acid exposure monitored immediately above the SCJ in patients and asymptomatic volunteers.

Table 19: Characteristics of esophageal acid exposure measured immediately above the SCJ insubjects with different ZAP grades.

	ZAP grade 0 (*n*=40)	ZAP grade I (*n*=121)	ZAP grade II (n=38	*p*
% time pH < 4, total	1.1 (0.6-2.4)	6.9 (3.0-9.9)	14.3(9.0-16.1)	< 0.001
% time pH < 4.0, upright	1.5 (0.9-3.3	8.3 (4.3-13.4)	15.5 (8.0-20.7)	< 0.001
% time pH < 4, supine	0.2 (0.0-1.0)	1.6 (0.1-6.0)	8.2 (4.2-16.2)	< 0.001
% time pH < 4, postprandial	1.9 (1.0-3.4)	12.1 (5.4-18.9)	22.7 (9.9-28.1)	< 0.001
# reflux episodes/hour	1.0 (0.5-1.7)	3.0 (1.7-4.7)	5.4 (4.0-6.7)	< 0.001
Longest reflux episode (min	3.5 (2.0-8.5)	15 (7-24)	28 (16.8-38.5)	< 0.001
# reflux episodes > 5 min	0 (0-1)	5 (2-11)	14 (8-24)	< 0.001
% abnormal acid exposure	5.0% (2/40)	23.1% (28/121	92.1% (35/38)	< 0.001

ZAP: Z- line appearance; SCJ: Squamocolumnar Junction

In individuals with ZAP grade 0, distal esophageal acid exposure was characterized by few reflux episodes with short duration, and the median % time spent below pH 4.0 was well within normal limits. The frequency and duration of reflux episodes as well as the degree of distal esophageal acid exposure increased significantly and progressively with increasing ZAP grade. Abnormal distal esophageal acid exposure was a rare finding in subjects with ZAP grade 0 but increased significantly with increasing ZAP grade. In order to study the effect of the severity of reflux disease on the geometry of the SCJ, subjects with abnormal esophageal acid exposure were divided into two equally large groups. As the median % time with pH below 4 in these patients was 10%, patients were classified as having moderate acid reflux if the % time with pH <4.0 was between

5.7 and 10.0 and as severe if it exceeded 10%. Figure 10 shows the distribution of ZAP grades in subjects based on the severity of esophageal acid exposure.

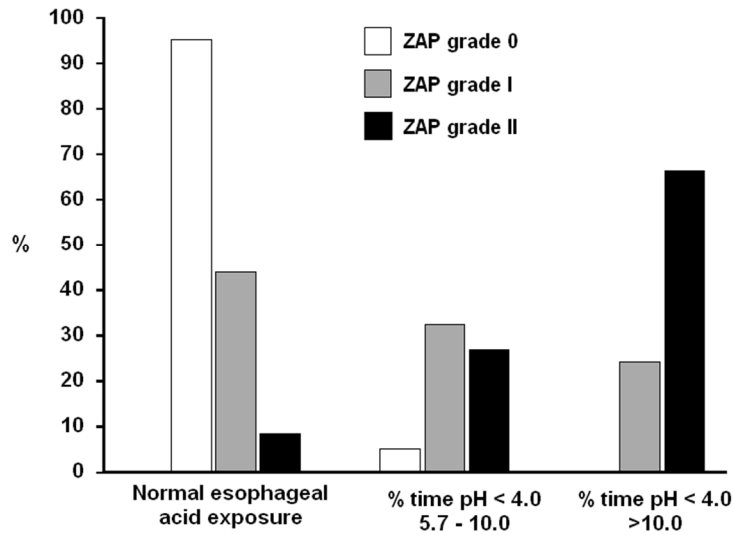


Figure 10: Distribution of ZAP grades in individuals with normal esophageal acid exposure, moderately increased acid exposure, and severely increased acid exposure measured by pH monitoring immediately above the scuamocolumnar junction.

There was a significant and progressive increase in the frequency and degree of irregularity of the SCJ from patients with normal acid exposure to those with moderate and severe acid exposure. In patients with severe acid reflux, ZAP grade II was the dominating finding and none of these patients were found to have ZAP grade 0. Table 20 shows the specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) for abnormal esophageal acid exposure for the different ZAP grades and for erosive esophagitis.

Table 20. Sensitivity, specificity, positive and negative predictive value for abnormal esophageal acid exposure in patients with varying ZAP grades and for the presence of esophagitis

	Sensitivity	Specificity	PPV	NPV
ZAP grade 0 (n=15)	2.0	73.5	0.130	0.269
ZAP grade I (n=99)	64.0	28.6	0.646	0.280
ZAP grade II (n=35)	34.0	96.0	0.971	0.280
Erosive esophagitis (n=58)	35.1	91.8	0.800	0.494

ZAP: Z-line appearance; PPV: predictive value of positive test;NPV: predictive value of negative test.

Interestingly, the sensitivity of erosive esophagitis and ZAP grade II was similar but the specificity and PPV were numerically higher for ZAP grade II, suggesting

that ZAP grade II may be a good predictor of reflux disease in patients with symptoms suggestive of GERD. The endoscopic appearance of the SCJ was graded according to the ZAP-classification and correlated with clinical characteristics and the pattern of acid reflux in the most distal esophagus in 50 asymptomatic healthy volunteers and 149 patients with symptoms suggestive of GERD. Even SCJs without irregularities were significantly more prevalent in asymptomatic volunteers compared with patients and were never found in patients with esophagitis. The median degree of distal esophageal acid exposure in individuals with an even SCJ was within normal limits. With increasing degree of irregularity of the SCJ, the frequency and duration of reflux episodes, the degree of distal esophageal acid exposure, and the prevalence of abnormal acid exposure increased progressively and significantly.

Discussion

In the management of patients with suspected GERD ambulatory pH monitoring represents the most widely used objective method evaluate acid exposure. Due to methodological limitations it has been necessary to position the pH sensor of the pH catheter 6 cm above the LES, but the optimal position is not known. Conventional catheter based pH monitoring has limitations, which to some extent has been avoided with the introduction of the wireless catheter system. The wireless pH system allows for less patient discomfort, and extended monitoring period and less interference with activities but without any improvement of diagnostic precision and still performed at the conventional level. Regardless if the pH test is performed with the catheter based or the wireless monitoring technique the diagnostic accuracy is relatively poor with a limited clinical value especially in patients without mucosal injuries and atypical symptoms.

The sensitivity and specificity of catheter based pH monitoring in identifying GERD is 87-96% and 97%-100% respectively and dates back 30 years (143, 146, 152), based on studies performed in patients with typical symptoms and severe reflux disease esophagitis, with defective LES. When erosive esophagitis is detected in these patients the diagnosis is clear (110) but in the majority of patients with suspected GERD without mucosal injuries and competent LES the sensitivity of the pH test drops to 36-48%, which substantially limits the clinical value. As patients with non-erosive disease constitute two thirds of all patients with reflux disease an improved diagnostic accuracy would be of great benefit in the management in patients with GERD. Previous studies have indicated that conventional pH monitoring substantially underestimates distal esophageal acid exposure and that only approximately 25% of detected distal reflux events reach the level of the conventional placed pH sensor, which could imply that the diagnostic precision could possibly be improved by a more distal placement of the sensor (134). Wireless pH monitoring permits a targeted more precise and consistent distal placement of the pH sensor. Despite this knowledge the positioning has remained at the conventional level, which encouraged us to study the diagnostic accuracy of pH monitoring by comparing wireless pH monitoring performed at the conventional level with that of immediately above the SCJ. It is also highly probable that the acid milieu and the pathophysiological mechanisms of GERD and complications, which most commonly occur in the area of the GEJ, are more optimally studied with a placement of the pH sensor as close to the SCJ as possible.

Diagnostic performance of pH monitoring in the most distal esophagus and symptom correlation

When introducing a new technique in clinical practice it is necessary to evaluate the diagnostic performance of the technique. The discriminative power and optimal values for parameters of esophageal acid reflux has been evaluated for wireless 48–h pH monitoring with normal values between 4.4 % and 5.3 % of the monitored time that pH is less than 4 (138, 141, 142). Variation in normal values is probably caused by such as differences in the inclusion of studied subjects, the heterogeneity of GERD, a lack of a clear definition between health and disease, lack of diagnostic gold standard methods of GERD and differences in methodology. In the evaluation of a diagnostic test it is important with a matched study population, a control group free from disease and that the patients with suspected disease really suffers from the disease. The combination of typical symptoms of reflux, such as heartburn and regurgitation, at least twice weekly with a distinct response to PPI therapy is highly suggestive of GERD (103, 125). We ensured a representative study population by exclusively including patients with typical symptoms of reflux with a minimum of reflux symptoms twice a week for 6 months prior to the study. Further we demanded a distinct response to PPI with reversibility upon termination of PPI. The group of patients without mucosal injury was reassessed with a 90 % symptom recurrence after PPI removal. Complications of reflux disease most commonly occur in the distal esophagus and it sensible to assume that positioning a pH sensor closer to the SCJ could reflect the acid environment better. As the diagnostic precision of pH monitoring is suboptimal for patients without mucosal injuries and previous studies has indicated that acid exposure is significantly underestimated at the conventional 6 cm level in the esophagus it is possible that the pH test could be improved by a monitoring position closer to the SCJ (134).

We were stimulated by innovative studies from Fletcher et al who performed pH recordings with a pH catheter equipped with two pH electrodes, fixed to the mucosa with clips and sutures, positioned at 0.5 cm above the LES and 5.5 cm above the SCJ, where they found a significantly higher acid exposure at the SCJ of patients previously with normal pH studies. We were further encouraged by the results from previous studies, on feasibility of pH monitoring close to the SCJ and that esophageal acid exposure is significantly underestimated at the conventional level compared with immediately above the SCJ (134). The acid environment in the most distal esophagus is thus practically unknown but of major importance in complications of GERD that most commonly occur in area of the GEJ. Reports also show an increasing trend of adenocarcinoma in the most distal esophagus with a pessimistic outcome with late detection (9, 21, 24, 79). Both of our pH monitoring studies was performed with simultaneous targeted pH monitoring

immediately above the SCJ and at the conventional level 6 cm above the SCJ and the pH data confirmed the results from Fletcher et al with at least twice the acid exposure at the SCJ site compared with the conventional site of pH monitoring. Although not presented, the analysis also showed an increased acid exposure in all parameters of acid exposure but particularly a doubled frequency of reflux events and events of long duration. The results are important as the true acid environment can reliably be characterized and established. Potentially this could help achieve more knowledge in the development of BE and adenocarcinoma of the esophagus.

As acid exposure is underestimated at the SCJ with conventional pH monitoring it is likely that also the diagnostic precision would be improved by a more distal recording. Performing ROC analysis is effective as an instrument to evaluate the diagnostic accuracy of parameters generated in a test, as the discriminative power of the test to separate abnormal from normal is directly related to the AUC curve (146, 170). We compared AUC values for different parameters of esophageal acid exposure, which served as an instrument to select the parameter with best distinguishing potential of the pH test. The total percent of time that esophageal pH was less than 4 for the entire 48-h, regardless of monitored level, generated the highest AUC values and consequently subsequently this was used for analysis of relevant cutoff level of specificity. Although both days had high AUC values the numeric difference was small, the highest AUC was generated for the combined entire 48-h period, which suggests that pH monitoring for 48 h should be utilized as far as possible. Pandolfino et al found the discriminative power of wireless pH monitoring was higher using the worst day of recording and similar to in previous studies, observed a day-to-day variability in acid exposure (138). However using the worst day of recording did not improve the sensitivity of the test in patients without mucosal injuries. These patients probably have a higher degree of day-to-day variability and would benefit more from a prolonged monitoring period (161). Independently of the parameter of acid exposure selected, lower AUC values was found in patients without mucosal injury and the highest AUC values were found in patients with esophagitis, suggesting that the discriminative power of the pH test increases with the severity of the disease.

The AUC reflects the diagnostic accuracy of all intervals of the curve of a test, including clinically irrelevant intervals. In both studies, the differences in AUC values were small but never the less it had a significant affect on the sensitivity and specificity. The inverse relationship between sensitivity and specificity may be used to compare the diagnostic performance of a test by selecting a predefined clinical applicable level of specificity. To be useful in clinical practice we believe 90 % specificity is needed and by comparing the sensitivity obtained at the both monitored levels with this fixed specificity, any difference of the test performed at the two different levels could be evaluated. The ROC analysis performed with 90% specificity, significantly increased the sensitivity in all groups in both studies

when performed immediately above the SCJ. The sensitivity of the test increased from 65 % to 84 % in all patients and improved between 19% and 24% from 78% to approximately 95% in patients with mucosal injury. Importantly, almost a 50 % improvement in sensitivity was seen in patients without mucosal injury, from a merely 45% to 67%. This is particularly interesting as patients without mucosal injuries represent a majority of GERD patients that often have a normal pH test at the conventional pH sensor position and consequently often are incorrectly diagnosed with functional disorders possibly withheld effective treatment (111, 171). A repeated consistent improved diagnostic accuracy of the pH test at SCJ in the second larger study confirms performing pH monitoring immediately above the SCJ improves diagnostic precision of the pH test. It could be argued that placing the capsule at such closeness to the SCJ could overestimate acid exposure, but several studies indicates such influence improbable (170, 172).

Our first study suggesting an improvement of diagnostic precision of pH monitoring in the most distal esophagus also raised the question if the temporal relationship between symptoms and reflux also would be stronger with the distal position resulting in an improved and clinically useful symptom association analysis. As heartburn and regurgitations in GERD are considered to be the most specific in symptoms of GERD, these were selected for the symptom association analysis. The SAP had the highest proportion of positive symptom analysis, indicating it superior as symptom association index, and was consequently selected for further analysis. The SAP being most representative and clinically useful of the symptom association indices has been suggested in other studies (173, 174). Due to fewer patients reporting symptoms regurgitations the symptom of heartburn was selected in the further analysis. Symptom associations immediately above the SCJ increased from 62% at the proximal 6 cm level to 76% in the distal esophagus. The likely explanation for a higher diagnostic yield of SAP at the SCJ is that indicated heartburn episodes by the patient now are matched with previously undetected reflux episodes. However, there is a slight possibility that more frequent episodes are matched by coincidence to symptoms. It has been indicated that heartburn is more frequently associated with a more distal reflux, which only to some extent could explain a difference in diagnostic yield of SAP with the proximal pH electrode. As plain pH monitoring often is insufficient for the objective GERD diagnosis and the definition of GERD also includes individuals without abnormal acid exposure that perceive symptoms from their reflux (3, 26) the combination of reflux monitoring and symptom association analysis is required to fully identify all patients with GERD. Thus, to explore the full potential of esophageal pH monitoring we assessed the diagnostic performance of the pH test at the two levels using the combination of abnormal esophageal acid exposure and/or a positive symptom association analysis as a

marker for a positive pH test. For the purpose of this, the SAP method was selected.

The discriminatory power of esophageal pH monitoring was significantly improved when SAP for heartburn was used as a marker for a positive pH test at the both monitored levels but with highest diagnostic yield at the distal site. Thus, the discriminatory power of the pH test was significantly improved with the pH electrode positioned in the most distal esophagus and it was further improved with the combination of SAP in all patient categories. Interestingly and important, the greatest improvement was seen in patients without mucosal injuries, who constitute the majority (two thirds) of patients with GERD. The predefined specificity of 90 % generated a sensitivity of pH monitoring at the conventional 6 cm level of a mere 46 %. By positioning the pH capsule with the electrode just above the SCJ, the sensitivity markedly increased to 66 % and with the combination of SAP as a marker for a positive pH test, the diagnostic yield further increased to 94 %. This observation has important clinical implications because it means that by simply placing the pH electrode further distally and using the results in combination with symptom association analysis, the diagnostic yield and the clinical value of esophageal pH testing improves markedly.

Noteworthy is that at 90 % specificity within the ROC curve, the generated cut off values for the % time that pH was less than 4 for the 48-h monitored period varied between 5.0 % and 5.7 % at the level immediately above the SCJ in the two studies. This is explained by the fact that more patients were included in the later study, changing the point for 90 % specificity within the ROC-curve, which generated a slightly higher cut off value. The somewhat lower sensitivity and specificity in patients with mucosal injuries in our studies compared with previous results is probably explained by patient selection with fewer patients with milder form of esophagitis, LAC A or B. In our studies the presence of esophagitis was 52% and 57%. A potential problem with positioning the pH capsule close to the SCJ is the risk of a capsule dipping below the SCJ recording gastric acid. We believe all pH electrodes in our study recorded esophageal acid exposure as attachment of the capsule was endoscopically overlooked and pH recordings were manually controlled for esophageal pattern, characterized by a baseline pH above 4-5. A pattern of gastric pH tracings is easily identified on the pH tracings.

Positioning the pH capsule immediately above the SCJ is technically more demanding due to movements of the GEJ and the contraction of the distal esophagus within the LES but with increasing experience of the examiner it is marginally more difficult. A significantly improved diagnostic precision of the pH test justifies the extra effort and difficulty.

We performed simultaneous targeted wireless pH monitoring immediately above the SCJ and compared acid exposure with that obtained at the conventional

proximal esophageal position. The main results are that the degree of acid exposure is significantly underestimated at the conventional level. Further, the diagnostic accuracy in pH monitoring and symptom analysis is significantly improved by a placement of the pH sensor close to the SCJ but importantly that the combination of pH monitoring and symptom association analysis performed in the most distal esophagus markedly increases the clinical value of the esophageal pH test.

Our observations suggest that pH monitoring with the pH electrode positioned immediately above the SCJ is superior to that performed at the standard level. It is a valuable tool in the clinical management of patients with reflux symptoms and should always be combined with symptom association analysis.

The acid pocket

The association between GERD and acid reflux is firmly established. Complications of GERD most commonly affect the most distal esophagus and not surprisingly, due to the close proximity to the stomach, acid exposure is higher in the distal esophagus. Therefore the acid environment in this region is paramount in the pathogenesis of GERD. The acid environment of the esophagus has previously been studied but the acid exposure of the most distal esophageal has been difficult to assess due the necessary placement in catheter based pH monitoring with the sensor in the proximal esophagus. Due to body movements, breathing and the shortening of the esophagus during swallowing causing undesirable movement of the un-fixed pH catheter, to pick up gastric acid if dipping into the stomach, it has been necessary to position the pH sensor in the mid esophagus. In an innovative study, securing the pH catheter to the mucosa of the distal esophagus with means of a metal clip, Fletcher et al demonstrated a significantly greater acid exposure close to the SCJ compared with the proximal 5 cm esophageal level (135). The same group performed pull through studies from the stomach across the GEJ into the esophagus and demonstrated the existence of a highly acidic segment of gastric juice in top of the stomach escaping the buffering effects of food. This segment, termed “the acid pocket”, was shown to extend proximally 2 cm into the distal esophagus for extended periods (69) and was proposed to be the source of postprandial acid reflux, which has been suggested to be an important factor in the pathophysiology of reflux induced injuries of the distal esophagus (175). The acid pocket is most likely the source of postprandial reflux events and many well-designed studies (68, 176-180) have confirmed the formation of un-buffered acid pocket in top of the stomach that transverses the GEJ.

Our interest was stimulated by the observation that the acid pocket was suggested to transverse the GEJ and last up to 90 minutes postprandially, exposing the distal esophagus to gastric acid for prolonged periods. If the theory should hold true, a distal pH sensor, such as the pH capsule immediately above the SCJ in our studies, would pick up a significant magnitude of prolonged postprandial acid exposure. PH recordings below the SCJ in patients with symptoms suggestive of reflux has demonstrated a pH below 4 the entire circadian circle in the most proximal stomach. Consequently the importance of the acid pocket must lie in the fact that it crosses the SCJ exposing the squamous mucosa to un-buffered acid for prolonged periods. The 48-h wireless pH recordings performed under physiological conditions with the pH sensor fixed just above the SCJ in our large group of reflux patients would be ideal to characterize the pattern of acid exposure in the most distal esophagus, which could elucidate the significance of the postprandial acid pocket in GERD. An acid pocket extending into the distal esophagus for long periods after meals would literally bath the distally placed pH sensor in gastric

juice from the acid pocket reservoir, with continuous acidic recordings. The recorded time with pH below 4 in patients with typical symptoms in our study was indeed significantly longer during the postprandial periods compared with the preprandial periods of reflux. However, the pattern was characterized by numerous short reflux events rapidly cleared from the distal esophagus. Long reflux events were actually significantly shorter in the postprandial period compared with the preprandial period and long reflux events lasting more than 5 minutes were rare. Reflux episodes longer than 5 minutes occurred only once every 2.8 h in the postprandial phase, which translates into reflex episodes longer than 5 minutes occurred once every second postprandial meal period in patients with severe reflux disease. With increasing severity of reflux disease the length of and the proximal border of the acid pocket seems to extend more proximally (68, 175, 179). In a majority of GERD patients (67%) with a hiatal hernia of larger size than 2 cm, the acid pocket had a location above the SCJ, which was suggested to explain why acid reflux occurs more often in patients with supra diaphragmatic hiatal hernias (68). Even though 93% of the patients with erosive disease in our study had hernia with a median length of 3 cm, the postprandial acid pattern was characterized by numerous short reflux events.

Studies describing the acid pocket are limited to small group of patients with studies performed in laboratory setting under non-physiological conditions (69, 176-178, 180). We believe our study is more representative for reflux in reality, with physiological 48-h pH recordings from the mucosal fixed distal wireless pH sensor in a large healthy control group and a large group of patients with typical symptoms of reflux. It is also not very likely that the pH sensor did not detect long acid reflux events from an acid pocket above the GEJ, as the pH electrode is very sensitive to changes in pH and samples every 6 seconds. The only reasonable explanation to the relative absence of long reflux events in our study is that gastric juice carried by the acid pocket does not transverse the GEJ for extended periods. Reasonable explanations to the contrasting observations from series of well designed studies probably lies in the methodology where the pull trough pH catheter could carry acid into the esophagus or possibly facilitate the proximal movement of gastric juice up together with the catheter.

Prolonged exposure of acid from the acid pocket is not likely sufficient to cause injuries to the esophagus but it is probably necessary with a reasonable high acidity for injuries to occur. The acidity of the acid pocket has been described with a median pH of 1.6, which is potentially very injurious as it potentiates the harmful effects from other gastric contents, such as pepsin being most proteolytically active at pH<3 with an increasing conversion at lower pH (66, 181). Bile in the combination of acid is also most aggressive at lower pH. If the acid pocket as proposed would be the source of postprandial reflux it would be reasonable to expect a similar pH as reported in the acid pocket. However the

median nadir pH of reflux events during the postprandal periods were relatively high and significantly higher than the preprandial periods. Further, the proportion of reflux episodes with a pH less than 2 or 3 was significantly fewer in the postprandial periods. This is consistent the observation made by Pandolfino et al who in simultaneous pH monitoring found a nadir pH almost invariable less in the cardia compared with the esophagus (182). The difference in medium pH observed in acid pockets and the minimum pH of reflux events observed in our study is incompletely understood but could possibly be explained by that the gastric refluxate, as it enters the esophagus, is gradually neutralized from swallowed saliva containing bicarbonate leading to a gradual increase in pH proximally along the esophagus.

The "acid pocket" theory states that a pocket of highly acidic gastric juice is formed in the most proximal stomach following meals and as laboratory studies indicate that the gastric pocket is a static phenomenon that extends into the distal esophagus for up to 90 minutes following a meal, it has been suggested to be an important factor in the pathophysiology of GERD and its complications. If the acid pocket, as described, was a static phenomenon lasting up to 90 minutes after meals, long repeated reflux episodes would have been expected, which we did not observe. In contrast, postprandial reflux was characterized by numerous short reflux events of a relatively harmless pH.

Although it is likely that the short reflux events detected in the most distal esophagus during postprandial periods are derived from the acid pocket, our observations suggest that the acid pocket phenomenon is confined to the proximal stomach. Multiple short reflux events are necessarily not abnormal as physiological acid reflux is characterized by rapidly cleared reflux episodes occurring in the upright position most commonly after meals. In contrast to the acid pocket theory, determinants of severe reflux disease and its associated complications generally include a defective lower esophageal sphincter, the proximal extension of the refluxate, poor esophageal clearance and most importantly prolonged exposure of the esophagus to gastric juice (44, 65). As the postprandial period only constitutes a small proportion of the entire circadian circle and the reflux during these periods were characterized by multiple short events with a relatively harmless acidity the acid pocket is probably of minor importance in the pathophysiology in GERD.

Our main observation was that postprandial acid exposure is characterized by numerous short reflux events with a relatively high pH and a rarity of long acidic reflux events. The relative absence of prolonged reflux events in the most distal esophagus suggests that the acid pocket is confined to the stomach and questions the damaging potential of the acid pocket and it´s importance in the pathophysiology of GERD and it´s complications.

The pathophysiology of acid reflux at the gastroesophageal junction

Metaplastic changes and injuries to the squamous mucosa most commonly occur in the most distal esophagus in the area of gastroesophageal junction (GEJ) at the SCJ, but the mechanisms underlying the pathological processes is largely unknown(183). The transition between the squamous epithelium of the esophagus and the columnar gastric mucosa is normally located in the area of the GEJ but surprisingly few studies exist on the normal appearance and in the shape of the SCJ and associations to the cellular composition in health and disease. Further the normal histological structure and the pathophysiology of cellular changes of the cardiac mucosa in the transition zone between the distal esophagus and the gastric fundus is debated. In available descriptions, the normal SCJ is defined as a sharp, serrated jagged line with short tongues of columnar mucosa extending up into the pink-white squamous epithelium of the esophagus (184-186). With a proximal extension of a salmon coloured mucosa regardless of segment length, tongues or irregularities from the proximal gastric folds to the SCJ, metaplasia should be suspected. Intestinal metaplasia (IM) of the columnar lined esophagus is strongly associated with reflux disease and adenocarcinoma, which has seen a rapid incline in incidence in the last decades (8-10). At the same time the prevalence of IM in the distal esophagus has been shown to correlate to the appearance of the SCJ (187).

IM in the stomach is associated with adenocarcinoma of gastric cancer, usually secondary to Helicobacter Pylori infection, but IM also appears frequently in an endoscopically apparent normal SCJ, with unclear clinical importance and etiology (85). It is generally accepted that reflux disease lead to inflammation and IM within the esophagus and that HP infection often lead to inflammation and subsequently atrophy and IM within the stomach. However, IM at the junction between these organs in the absence of endoscopically apparent metaplastic columnar mucosa is controversial. The results of available studies are conflicting with reported associations with both H. Pylori infection and with gastroesophageal reflux (183, 188-195). In studies on associations between IM at the SCJ and Helicobacter Pylori (HP) or gastroesophageal reflux are based on symptomatic patients and presumably asymptomatic individuals but without any objective documentation of acid exposure, similar to the studies on the shape of the SCJ (188-192, 195, 196). The acid environment in the area of the GEJ is of particular importance in the studies of associations between complications, such as esophagitis, IM and adenocarcinoma and reflux disease. However, information of the acid milieu in the area of the GEJ has until recently been sparse as the pH electrode of traditional catheter-based techniques for esophageal pH monitoring are positioned 6-7 cm above the SCJ in order to avoid gastric pH tracings (197,

198). The distally targeted pH sensor close to the SCJ is ideal and provides necessary information on the acid environment to reliably study associations between acid reflux, metaplastic changes and appearance of the SCJ in the pathophysiology of GERD. Complemented with analysis of HP infection, important knowledge to help clarify the etiology of IM at the SCJ could be obtained.

We studied the etiology of IM at the GEJ by histologic examination of biopsies obtained from the exact level of SCJ, by targeted recording of acid reflux immediately above the SCJ in asymptomatic volunteers and symptomatic patients. As most commonly in clinical practice a circumferentially even SCJ with minimal tongues of irregularities, corresponding to the ZAP grades 0 and I, is considered normal these were selected for the purpose of biopsies of the normal SCJ. Most previous studies on the etiology of IM at the GEJ have included subjects with an endoscopically normal appearing SCJ postulating normality without any scientific basis or a distinct descriptive appearance. The importance of using a standardized assessment protocol is supported by the differences in results of previous studies, which further makes conclusions difficult and results incomparable. We believe our results are reliable and possible to reproduce as we used a systemized assessment, the ZAP grade, for better demarcation and distinct sampling of the SCJ allowing for comparison in future studies. The observed prevalence of IM at the normal appearing SCJ (ZAP 0 and I) was 28% (45/161) with no difference in prevalence between asymptomatic volunteers and patients. Several other studies have reported similar results but there is significant variation (23-44%) in prevalence of specialized intestinal metaplasia at the SCJ (193, 199, 200). The most probable explanation in differences in prevalence between studies is the use of different biopsy protocols, non systemized assessment and possibly the technique in obtaining biopsies such as the forceps overbridging the SCJ, retro ward or purely esophageal sampling (201). The prevalence of HP infection in our study was 7%, which is considerably lower than that of similar studies (188-196, 202). We believe the prevalence of HP is reliable, although somewhat low, as presence of HP infection was ensured both with analysis of faecal antigen test and in biopsies from the stomach. A possible explanation is a low incidence of HP in Sweden compared with other parts in the world and a selection bias with differences in HP infection in the different study populations. In contrast to several studies no association between IM at the SCJ and HP infection was found.

The fact that the term gastric cardia is used both as the anatomical area of the most proximal part of the stomach as well as for the extremely short segment gastric cardiac mucosa is confusing and misleading, resulting in misconception and may well explain contradictory results. The true gastric cardia containing cardiac mucosa is a transition zone (<0.4 mm) adjacent the distal esophagus and the proximal stomach histologically containing mucous or sometimes mixed mucous

and oxyntic glands (40, 203). Our biopsies were obtained with the jaws of the forceps on either side of the SCJ while in many studies the biopsies has been obtained well below the SCJ, which means they are of gastric origin and composed of mucosa resistant to acid. It is therefore not surprising that IM and inflammation in those biopsies is associated with HP and not the hallmarks of GERD. It is however possible that IM in the biopsies of cardiac mucosa, which appears to be affected by the injurious effect of both gastric acid and HP infection, may have two distinct different etiologies. It may be the consequence of acid reflux as suggested by our results or also conceivably secondary to inflammation in patients with HP infection.

Our results suggest that IM at a normal appearing SCJ is a consequence of gastroesophageal reflux. The strongest predictor for IM at the GEJ in the binary logistic regression analysis was the presence of abnormal acid exposure monitored immediately above the SCJ, with a relative risk that was 5.5 times higher than for subjects with normal acid exposure. The number of reflux episodes per hour was also associated with an independent and statistical significant increase in relative risk, a risk that increased 1.5 times with every single reflux episode/hour. The finding of IM in individuals with an acid exposure within normal limits is intriguing and was somewhat unexpected. There can be several explanations worth considering. There is a very slight possibility that the monitored acid does not reflect actual exposure due to the diagnostic accuracy of the method or that in these individuals the pathological process has started earlier as a consequence to previous insults from abnormal acid exposure or possibly an unknown provocative. It could also be explained by a variable predisposition to develop IM in response to injury. IM at an endoscopically normal GEJ could be the consequence of physiological reflux or moderate reflux at the level of the SCJ in subjects prone for the development of metaplasia. Subjects with such a predisposition and a higher degree of reflux that extends further into the esophagus would most likely develop endoscopically visible segments of columnar metaplasia. These hypotheses are supported by studies suggesting that there may be an individual predisposition for the development of metaplasia and that the extent of esophageal metaplasia correlates with the degree of esophageal acid exposure (43, 44).

Observations of IM at a conceivable normal SCJ also bring questions on what is the appearance of a normal SCJ and possible associations between differences in shape and disease. Our interest was also initiated by observations of few irregularities of the SCJ in asymptomatic individuals in contrast to patients with reflux symptoms. Wallner et al studied the SCJ for association between the shape of the SCJ and reflux disease and introduced the Z-line appearance (ZAP) in an attempt to systemize assessment of the shape of the SCJ. The ZAP classifies the geometry of the SCJ in four grades (166). Their studies were based on symptoms

in patients with suspected reflux and patients considered asymptomatic, as they were examined for other reasons than suspected GERD, but the studies lacked objective evaluation of acid exposure. This and the fact that the distal placed pH sensor identifies a significantly higher quantity of reflux episodes likely to more reliably characterize the acid reflux pattern in the most distal esophagus, inspired us to study association between shape of the SCJ with GERD.

Studies using simultaneous dual pH monitoring with one capsule positioned with the pH electrode at the traditional level and one immediately above the SCJ, has demonstrated that the degree of acid exposure at the level of the GEJ is substantially underestimated by traditionally placed pH electrodes and that only approximately 25% of reflux events detected at the level of the SCJ extend to the level of conventionally placed pH sensors. In our study in the shape of the SCJ, the observations allowed us to conclude that the normal SCJ is circumferentially even without any serrations. This is important because there previously did not exist a clear definition of normality, which is necessary for the defining abnormality and disease. The ZAP grade 0 of the SCJ was the dominating finding in asymptomatic volunteers and observed in only 3% of patients with typical symptoms of reflux, an observation indicating that a normal SCJ is most likely appearing as even without any serrations. This is supported by the observation that ZAP grade 0 was exclusively observed in patients without mucosal injuries and not at all in patients with erosive esophagitis. Further, abnormal distal acid esophageal environment wouldn't be expected at a normal SCJ and the observation that median degree of distal esophageal acid exposure was well within normal limits in subjects with ZAP grad 0 clearly strengthens our conclusion. The individuals with ZAP grade 0 also elicited a typical physiological reflux pattern, characterized by few short reflux episodes predominately in the postprandial upright position periods. If irregularities were formed in response to chronic acid injuries, such as other acid related complications, then an progressive increase in both frequency of mucosal injuries and acid exposure would be followed by increase in ZAP grade.

It is interesting that although most symptomatic patients with abnormal acid exposure had irregular SCJs there were a small proportion of symptomatic patients without any irregularities. Despite improvement of the pH test with more distal performed pH monitoring the diagnostic precision is not entirely reliable, which possible may lead to both under- as well as overestimation of actual acid exposure that could explain both the finding of patients without irregularities at the SCJ and abnormal acid exposure and patients with irregularities and normal acid exposure. It is also possible that these contradictory observations may be explained by an individual susceptibility or predisposition to develop columnar metaplasia not entirely dependable on the acid exposure. This hypothesis is supported by the observation that patients who undergo esophagectomy and gastric tube reconstruction for Barrett´s related adenocarcinoma develop metaplasia in the

remaining cervical esophagus more frequently that patients who are subjected for esophagectomy for other reasons(43). The controversies on the management of BE are affected by prevalence of BE affected by performed endoscopies, biopsy taking, sampling error, length of the metaplastic segment and the relative risk incidence in developing BE associated adenocarcinoma. Our observations suggest that also minimal irregularities of the SCJ constitute small areas of metaplastic columnar mucosa and the current definition of GERD, which includes complications in both asymptomatic and symptomatic individuals. Clearly, if irregularities as we suggest is a complication from acid reflux then this will mean that 50 % of the asymptomatic healthy population should also be considered having GERD. It is not practical that a definition includes such a large proportion of the whole population with potentially only a minimal risk of serious complications. A revision of the definition of GERD seems reasonable(3).

If 95% of individuals with a SCJ without irregularities have normal distal esophagel acid exposure it raises the possibility to use ZAP 0 as a predictor for normal acid exposure and similarly the ZAP grade II, with 92 % of the patients with abnormal acid exposure, used as a predictor for reflux disease. In or analysis of sensitivity, specificity, positive and negative predictive value the sensitivity of ZAP grade II was similar to erosive esophagitis in predicting abnormal acid exposure but the ZAP II had a higher specificity. This is interesting in the hands of the endoscopist to use endoscopic appearance of the SCJ to possibly predict GERD and it is supported by the fact that PPV was markedly higher for ZAP grade II than that for erosive esophagitis. It may thus also besides for the use in studies have important clinical implication in using a standardized classification system for the evaluation of the SCJ. Three experienced examiners participated in our study and had similar evaluations in re-evaluated cases. Further the ZAP grade classification has been evaluated for the assessment of the SCJ and the reported intra- and interobserver reproducibility is high. The result is interesting and possibly could be used in the diagnosis of GERD in clinical practice in the hands of the endoscopist. However, the result needs to be confirmed and evaluated in more studies.

We believe our effort in characterizing the geometry of the SCJ in a standard fashion in order to exclude subjects with clearly irregular SCJs (ZAP II and III) contribute to the credibility of IM at the normal SCJ and the association with ZAP 0 and I. IM at the GEJ in biopsies of the SCJ in subjects with ZAP grade I was significantly more frequent than in subjects with an entirely distinct even SCJ, which emphasizes the importance of a distinct classification in studies of this kind. Our observation also confirms that also small irregularities of the SCJ constitute small areas of metaplastic columnar mucosa and manifestations of acid reflux. Subjects with ZAP grade 0 were rare, which makes analysis of that group difficult. In a subanalysis of subjects with a SCJ corresponding to ZAP grade 0, no

significant association between IM and acid reflux or HP infection could be found. Although it is probable that IM at the GEJ is associated with a higher risk of developing adenocarcinoma, IM at the normal SCJ is a frequent finding and the risk of adenocarcinoma in BE is associated with increasing length of the metaplasia(204). Therefore the risk of adenocarcinoma in minimal areas of metaplasia at the SCJ is most likely negligible for the individual patient and consequently routine biopsies or protocols for surveillance will probably not be efficient and cannot be recommended.

Our main result in these studies is that the normal squamocolumnar junction is circumferentially even and even these minimal irregularities of the SCJ represents small areas of intestinal metaplasia as a consequence from acid reflux. Further IM of the SCJ is a consequence of gastroesophagel reflux disease and not related to HP infection.

Conclusions

I. The degree of acid exposure in the most distal esophagus is significantly underestimated using the conventional pH electrode position. Wireless pH monitoring immediately above the SCJ is feasible and may increase the diagnostic accuracy of esophageal pH monitoring compared with the standard electrode placement.

II. The diagnostic accuracy of esophageal pH monitoring in the most distal esophagus is confirmed superior to that performed at the conventional level and it is further improved with the combination of symptom association analysis. The pH test immediately above the SCJ is a valuable diagnostic instrument and should always be combined with symptom analysis.

III. Postprandial acid exposure is characterized by numerous short reflux events with a relatively high pH and the relative absence of long acidic reflux events suggests that the acid pocket is confined to the stomach and questions the importance of the "acid pocket" in the pathophysiology of GERD.

IV. IM at the GEJ in a normal appearing SCJ, corresponding to ZAP grade 0 and I, is a consequence of gastroesophageal reflux and not associated with HP infection. The clinical importance of this entity is unclear and warrants further studies.

V. The normal SCJ is even and without irregularities, corresponding to ZAP grade 0. Also minimal irregularities of the SCJ are acquired and most likely constitute small areas of metaplastic columnar mucosa that are formed in response to chronic injury from acid reflux. The geometry of the SCJ provides information that may be a useful in the management of patients with symptoms suggestive of GERD.

Future studies

The gastroesophageal junction is the most common area for complications of reflux disease, but the acid environment and the pathophysiology of gastroesophageal reflux and its complication needs further investigation.

Hiatal hernia is common in patients with reflux symptoms but it is also found relatively frequently in asymptomatic individuals. The severity of reflux disease has been reported to be correlated to the size of the hernia, but the pathophysiological mechanisms are not entirely clear. In an attempt to further study the pathophysiological mechanism of GERD for we plan to study the effect of hiatal hernia, the size of the hernia and the geometry of the area of the gastroesophageal junction (Hill grade) on the pattern and the degree of acid reflux, at the level of the SCJ and in the mid esophagus.

The mechanisms by which reflux of gastric juice into the esophagus elicit reflux symptoms are incompletely understood and it is not known why some reflux episodes are symptomatic while others are not. We therefore plan to study the pattern, the degree and the height of acid reflux in patients with different reflux symptoms such as heartburn, regurgitation and dysphagia. We hypothesize that patients with regurgitation have more severe reflux than patients with other symptoms suggestive of reflux disease. We also attempt to study the temporal association between acid reflux events at different levels of the esophagus and symptoms in order to characterize reflux events that lead to symptoms.

Or something completely different…..

Populärvetenskaplig sammanfattning

Gastroesofageal refluxsjukdom (GERD) är en folksjukdom där cirka 20 % av befolkningen återkommande flera gånger per vecka upplever de typiska reflux symptomen bröstbränna och sura uppstötningar. Reflux symptom upplevs av cirka 40 % av befolkningen varav matstrupsinflammation förekommer hos upp till 20 % av befolkningen och endast 2/3 av dessa uppvisar symptom. Gastroesofageal reflux innebär att surt maginnehåll ifrån magsäcken backar onormalt upp i matstrupen, vilket kan utlösa symptom eller kan förekomma helt utan symptom men ändå orsaka skador. Magsaften innehåller bl a saltsyra som tillblandas med galla, proteiner och andra ämnen från tolvfingertarmen vars huvudsakliga uppgift är att spjälka nedsvald föda. Syran kan ge upphov till olika symptom och kan i sig orsaka skador men även aktivera spjälkande proteiner i sur miljö vilket kan orsaka förvärrad skada på matstrupens slemhinna, vilken till skillnad från magsäckens slemhinna inte är motståndskraftig mot syra och spjälkande proteiner. Normalt kan kroppen via försvarsmekanismer hantera syra angrepp men vid reflux sjukdom förkommer flera defekter i barriär försvaret.

Återkommande symptom av bröstbränna och sura uppstötningar har en påtagligt negativ påverkan på patienter i sitt dagliga liv med en betydande försämrad livskvalitet jämförbar med kärlkramp och hjärtsvikt men den är också sämre än patienter med diabetes och hjärt- kärlsjukdom. Individer med reflux symptom söker ofta och vanligen först till primärvården för sina symptom och förskrives vanligen syra hämmande läkemedel som minskar magsyraproduktionen både i diagnostiskt såväl som terapeutiskt syfte. Hos yngre patienter utan misstanke på allvarlig sjukdom överensstämmer ofta symtom med svårighetsgraden av syra exponering i nedersta delen av matstrupen och de behandlas ofta följaktligen med syrahämmande läkemedel utan initiering av utredning. Det finns också en omfattande egenbehandling med receptfri syrahämmande läkemedel av reflux symptom i samhället. Gastroesofageal reflux sjukdom är kronisk och patienter som lider av sjuklig reflux har oftast god effekt av syrahämmande medicinering, men vid uppehåll eller avslutande av behandlingen återkommer symptomen i princip alltid. Beroende på en hög sjukdomsförekomst, hög läkemedelskostnad, sjukfrånvaro, stort antal sjukbesök och minskning av arbetsproduktivitet är samhällskostnaden för reflux sjukdom mycket hög och beräknades kosta samhället runt 3 miljarder SEK 1997. En väldigt liten del av utgifterna utgörs av

utredningskostnader för sjukdomen och en stor del av patienter behandlade med syrahämmande läkemedel saknar klar diagnos och har inte refluxsjukdom.

Reflux av magsaft till matstrupen kan orsaka skador såsom inflammation, sår, förträngningar, blödning, cellförändringar av matstrupens normala skivepitel till cylinderepitel, precancerösa förändringar, s.k Barretts esofagus (BE) och matstrupscancer s.k. adenocarcinom. Den allvarligaste komplikationen utgörs av adenocancer i matstrupen som ökat rekordartat och är en av de snabbaste ökande cancerformerna i västvärlden med dålig behandlingsprognos särskilt om den upptäcks sent. Det finns ett starkt etablerat samband mellan reflux sjukdom, BE och adenocarcionom i matstrupen.

Individer med reflux symptom och särskilt de som inte har effekt eller otillräcklig lindring av syrahämmande behandling utreds vanligen med s.k. gastroskopi (kikarundersökning) och ibland även med manometri (tryckmätning) och pH mätning. Patienter med svår reflux sjukdom blir ofta föremål för vidare utredning då en eventuell kirurgisk rekonstruktion för att återställa barriärfunktionen i nedre delen av matstrupen övervägs. Vid fynd av inflammation i matstrupen i kombination reflux symptom är ofta diagnosen GERD till stor del säker och ytterligare utredning är oftast inte nödvändig. Dock uppvisar majoriteten (75 %) av patienterna med reflux symptom inga synliga skador på matstrupens slemhinna, vilket medför diagnostiska utmaningar då pH mätning som bästa diagnostisk metod hos dessa patienter inte har bättre diagnostisk precision än att hos 60 % undersökta patienter identifiera sjuklig syra exponering. Vid pH mätning registreras syra koncentrationen av en mät sensor vilket kan ske med en pH elektrod i spetsen av en plast kateter eller av en trådlös kapsel med en pH elektrod. Katetern har kopplats till en portabel mottagare som registrerar pH värden i patientens vanliga hem eller arbetsmiljö under 24 timmar varefter data överförs till en dator för analys.

Traditionellt har pH registrering med kateter buren metod skett 5 cm ovan nedre matstrupssfinktern vars nedre begränsning lokaliseras före undersökningen med tryckmätning. Denna nivå är vald beroende på metodologiska problem där pH kateterns positionering kan förflyttas vid kroppslägesförändringar, andningsrörelser och fysiologisk sväljningsigångsatt förkortning att matstrupen, vilket kan leda till att pH sensorn doppar ner i magsäcken och registrerar syra ifrån magsäcken istället för i matstrupen. Den traditionella mät nivån avspeglar inte faktiskt syra nivå i nedersta delen av matstrupen och den optimala nivån för pH mätning är inte känd. Många patienter har upplevt fysiskt obehag av pH katetern förenat med socialt begränsning vilket sannolikt lett till att patienten undvikit vanliga dagliga aktiviteter vilket teoretiskt kan påverka mätresultaten. Med den trådlösa tekniken fästes pH kapseln i slemhinnan med ett stift och den kan placeras i valfri position i matstrupen. Positioneringen av den trådlösa pH kapsel har

kvarstått på traditionell nivå och trots mindre social och fysisk begränsning, minskat obehag från den trådlösa pH kapseln och förlängd mättid till 48 timmar är den diagnostiska träffsäkerheten oförändrad.

Huvudsakligen inträffar komplikationer till refluxsjukdom längst nederst i matstrupen i området mellan magsäck och matstrupen vid skivepitelövergången (SCJ) och då traditionell pH mätning sker långt ifrån där komplikationer till reflux sjukdom sker är refluxmönster och graden av syra exponering i detta område till stor del okänd. Det är numera visat att graden av syra påtagligt underskattas vid pH registrering vid traditionell 5 cm nivå jämfört med direkt invid skivepitelövergången och den trådlösa pH mätningsmetoden med fritt valbar oföränderlig positionering ger unika möjlighet att studera faktisk syra miljö och kopplingar till sjukdomstillstånd i nedersta matstrupen.

Avhandlingen syftar till att studera och karaktärisera den faktiska syra miljön med trådlös pH mätning utförd ovan nära invid skivepitel övergången i matstrupen och att jämföra diagnostiska precisionen av pH mätning utförd på denna nivå jämfört med pH mätning utförd vid den traditionella nivån i matstrupen. Vidare att studera sambandet mellan mönstret av sur reflux längst nederst i matstrupen och slemhinneförändringar vid skivepitelövergången i matstrupen.

Arbete I och II

Delarbete I och II syftade till att karakterisera syra miljön direkt invid skivepitelövergången i nedersta delen av matstrupen och att utvärdera pH mätning som metod utförd här för att korrekt diagnosticera patienter med refluxsjukdom jämfört med pH mätning utförd vid traditionell nivå 5-6 cm högre upp i matstrupen. På friska frivilliga och patienter med typiska reflux symptom utfördes samtidig pH mätning med en pH kapsel på vardera mät nivå där grad och mönster av sur reflux jämfördes mellan mät nivåerna.

Studien visade att pH registrering vid den övre mät nivån kraftigt underskattade graden av reflux längst ner i matstrupen hos reflux patienter såväl hos friska frivilliga. Resultaten visade att med ökande svårighetsgrad av reflux sjukdom ökar pH testets förmåga att korrekt ställa diagnos. Vidare visades i studie I att den lägre pH kapsel placeringen påtagligt förbättrade pH testets förmåga att korrekt identifiera patienter med reflux sjukdom både med och utan inflammation i matstrupen. Indikationen att pH testets förmåga att särskilja friska från sjuka individer med reflux sjukdom föranledde en upprepad större studie med fler inkluderade patienter med typiska reflux sjukdom. Båda studierna visade likvärdigt en förbättrad identifieringsförmåga av reflux patienter när pH registreringen sker vid den lägre mät nivån invid skivepitelövergången. Förbättringen var störst hos patienter utan inflammation i matstrupen, vilken är den patient kategori som är störst bland patienter med refluxsjukdom. Vidare

eftersom att testets diskrimineringsförmåga ökade med den lägre mätmetod nivån prövades också hypotesen att även symptom utvärdering skulle kunna förbättras vid analys vid den lägre mät nivån. Vid symptom analys kopplas patientens förmedlade symptom under pH registreringen med reflux episoder enligt olika symptom analys metoder. Den metod som hade bäst utfall var den s.k. symptom association probability (SAP) vilken prövades i pH testet och visade att även symptom analys av SAP för halsbränna förbättrades vid den lägre mät nivån. Vidare visade analysen att pH mätning i kombination av symptomanalys av SAP, som en markör för ett onormalt pH test, ytterligare ökade pH testets diskrimineringsförmåga av sjuka individer. Slutsatsen är att pH mätning bör utföras nära ovan invid skiveptielövergången och alltid i kombination med symptomanalys.

Arbete III

Innehållet i magsäcken skiktar sig så att syran lägger sig i översta delen av magsäcken, vilket har benämnts som syraficka. Studier har visat att syrafickan sträcker sig över magsäcksövergången upp i matstrupen och kvarstår där under långa perioder efter måltider och utsätter matstrupen för långvarig syra exponering. Detta fenomen har föreslagit ha stor betydelse i sjukdomsmekanismerna vid symptom och komplikationer av reflux sjukdom. Vi studerade och karakteriserade syramiljön i nedersta matstrupen genom pH registrering av en pH kapsel positionerad direkt ovan invid skivepitelövergången och jämförde mönster och grad av sur reflux i perioder 90 minuter efter måltid med perioder före måltid exkluderande själva måltidsperioden.

Studien visade att syra nivån var betydligt högre efter måltid än före måltider. Dock karakteriserades reflux mönstret av syra efter måltid av frekventa korta syra attacker av låg syra halt. Långvarig syra attacker var ovanliga. Den relativa avsaknaden av långvariga syra attacker efter måltid motsäger att syra fickan sträcker sig förbi magsäcksövergången upp i matstrupen under långa perioder efter måltid. Våra resultat ifrågasätter därför skade potentialen av ”syrafickan” och dess betydelse i sjukdomsmekanismerna vid reflux sjukdom.

Arbete IV

Intestinal metaplasi (IM), vilket innebär en omvandling av den normala skivepitel slemhinnan i nedersta delen av matstrupen till en slemhinna som i stort liknar magsäcksslemhinnan, är nära kopplat till reflux sjukdom och är förenat med en ökad risk att utveckla adenocancer i matstrupen. Orsaken till IM är debatterad och anses av en del vara orsakat av syra reflux och andra av Helicobakter infektion (HP). IM i den normala skivepitelövergången mot matstrupen (SCJ) är ett inte ovanligt fenomen men dess betydelse är oklar. Orsaken till IM har kopplats till reflux hos symptomatiska patienter men tyvärr med avsaknad av objektiv

utvärdering av syra exponering. Tidigare med den kateterbaserad pH mätningsmetoden kunde inte syra miljön längst ner i matstrupen invid SCJ där komplikationer till reflux sjukdom huvudsakligen uppstår inte studeras beroende på pH elektrodens nödvändiga positionering 6 cm ovan SCJ. Vår studie avsåg att med en pH kapsel placerad invid SCJ tillförlitligt utvärdera den sura miljön och undersöka samband mellan IM och sur reflux eller HP infektion. En studiepopulation med normalt utseende av SCJ undersöktes för samband mellan syra reflux och eller HP infektion och IM. Provbiopsier togs över SCJ för histologisk analys av IM. Närvaro av HP undersöktes med provbiopsier och med analys av förekomst av antigen för HP i avföring.

Resultatet av studien visade en relativt hög förekomst av IM. Vi fann inget samband mellan HP infektion och IM i matstrupen. Däremot var IM i matstrupen nära kopplat till onormal syra exponering. Individer med onormal syraexponering hade 5.5 gånger högre risk för IM än individer med normal syraexponering. Även antalet syraattacker var förenat med en ökad risk för IM.

Intestinal metaplasi vid skivepitelövergången är en manifestation av refluxsjukdom men inte av Helicobakter infektion.

Arbete V

Den normala skivepitelövergången (SCJ) även kallad Z linjen beskrivs ofta som välavgränsad sågtandsformad med enstaka korta, tungformade laxrosa projektioner som sträcker sig upp i matstrupen. Det är dock så att det inte finns någon klar vedertagen definition på hur en normal SCJ ser ut och påfallande få studier över detta finns. Studier har undersökt utseendet av SCJ hos patienter med misstänkt reflux sjukdom genom systematisk bedömning av SCJ via den s.k. ZAP (Z linje utseendet) men resultaten är inte helt tillförlitliga då objektiv utvärdering av syra exponering vid Z-linjen inte utförts. Vi undersökte ett stort antal patienter och friska frivilliga och kategoriserade deras skivepitelövergång efter ZAP klassifikationen och analyserade samband mellan syraexponering och ZAP hos patienter med atypiska typiska symptom och asymptomatiska frivilliga.

Resultaten visade att majoriteten av friska frivilliga har mindre ojämnheter i Z-linjen och att dessa oregelbundenheter ökade grad artat från patienter med atypiska symptom till patienter med typiska symptom men framförallt att oregelbundenheterna i Z linjen ökade dos artat med ökad syraexponering i nedersta delen av matstrupen. Vi konkluderade att den normala skivepitelövergången är jämn i formen och att oregelbundenheter är en konsekvens av syra reflux och sannolikt utgörs av små formationer av metaplastiskt förändrad slemhinna.

Acknowledgement

I would like to express my highest gratitude to:

Stefan Öberg, my supervisor and mentor, for initiating me in the scientific world of research and for patience with me in times of difficulties. For support and help and for the small talk concerning life.

And last but not least for damage control.

My co-authors: Jörgen Wenner for stimulating collaboration and encouraging words. Peter Scherman for collaboration in our studies.

My parents for their endless faith in me, support and encouraging words. Always believing…

Thor and Enya for just being the best children a parent could wish for and for patience and putting up with a dad at times not really there.

My brother Håkan for just being my little brother always to be counted on when needed. And the love of his life Margareta for encouraging words.

My ex wives for reminding me of what it´s all about: progress, evaluation, the future and happiness

Monica Keidser for help and guidance in the administration process of research

The Old Boys Hockey club for anger management.

The Classic racing series for more speed than in research, letting me take the heat off

Malmö endoscopy unit and colleagues for the pleasure of working at the same time having a laugh in times of difficulties and routine assignments

All colleagues at the department of Gastroenterology SUS

Södra Sjukvårdsregionen and Bengt Ihre fonden for supporting my research

References

1. Locke GR, 3rd, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ, 3rd. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Olmsted County, Minnesota. Gastroenterology. 1997;112(5):1448-56.
2. El-Serag HB, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut. 2014;63(6):871-80.
3. Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. The American journal of gastroenterology. 2006;101(8):1900-20; quiz 43.
4. sandler RS, Everhart JE, Donowitz M, Adams E, Cronin K, Goodman C, et al. The burden of selected digestive diseases in the United States. Gastroenterology. 2002;122(5):1500-11.
5. Peery AF, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, et al. Burden of gastrointestinal disease in the United States: 2012 update. Gastroenterology. 2012;143(5):1179-87 e1-3.
6. Van der Veen AH, Dees J, Blankensteijn JD, Van Blankenstein M. Adenocarcinoma in Barrett's oesophagus: an overrated risk. Gut. 1989;30(1):14-8.
7. Drewitz DJ, Sampliner RE, Garewal HS. The incidence of adenocarcinoma in Barrett's esophagus: a prospective study of 170 patients followed 4.8 years. The American journal of gastroenterology. 1997;92(2):212-5.
8. Devesa SS, Blot WJ, Fraumeni JF. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. Cancer. 1998;83(10):2049-53.
9. Brown LM, Devesa SS, Chow WH. Incidence of adenocarcinoma of the esophagus among white Americans by sex, stage, and age. Journal of the National Cancer Institute. 2008;100(16):1184-7.
10. Lagergren J, Bergström R, Lindgren A, Nyrén O. Symptomatic Gastroesophageal Reflux as a Risk Factor for Esophageal Adenocarcinoma. New England Journal of Medicine. 1999;340(11):825-31.
11. Bernstein LM, Baker LA. A clinical test for esophagitis. Gastroenterology. 1958;34(5):760-81.
12. Bredenoord AJ, Weusten BL, Timmer R, Smout AJ. Characteristics of gastroesophageal reflux in symptomatic patients with and without excessive esophageal acid exposure. The American journal of gastroenterology. 2006;101(11):2470-5.

13. Emerenziani S, Sifrim D, Habib FI, Ribolsi M, Guarino MP, Rizzi M, et al. Presence of gas in the refluxate enhances reflux perception in non-erosive patients with physiological acid exposure of the oesophagus. Gut. 2008;57(4):443-7.
14. Howard PJ, Maher L, Pryde A, Heading RC. Symptomatic gastro-oesophageal reflux, abnormal oesophageal acid exposure, and mucosal acid sensitivity are three separate, though related, aspects of gastro-oesophageal reflux disease. Gut. 1991;32(2):128-32.
15. Trimble KC, Pryde A, Heading RC. Lowered oesophageal sensory thresholds in patients with symptomatic but not excess gastro-oesophageal reflux: evidence for a spectrum of visceral sensitivity in GORD. Gut. 1995;37(1):7-12.
16. Reddy H, Staahl C, Arendt-Nielsen L, Gregersen H, Drewes AM, Funch-Jensen P. Sensory and biomechanical properties of the esophagus in non-erosive reflux disease. Scandinavian journal of gastroenterology. 2007;42(4):432-40.
17. Byrne PJ, Mulligan ED, O'Riordan J, Keeling PW, Reynolds JV. Impaired visceral sensitivity to acid reflux in patients with Barrett's esophagus. The role of esophageal motility*. Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus / ISDE. 2003;16(3):199-203.
18. Klauser AG, Schindlbeck NE, Muller-Lissner SA. Symptoms in gastro-oesophageal reflux disease. Lancet. 1990;335(8683):205-8.
19. Tefera L, Fein M, Ritter MP, Bremner CG, Crookes PF, Peters JH, et al. Can the combination of symptoms and endoscopy confirm the presence of gastroesophageal reflux disease? The American surgeon. 1997;63(10):933-6.
20. Armstrong D. Endoscopic evaluation of gastro-esophageal reflux disease. The Yale journal of biology and medicine. 1999;72(2-3):93-100.
21. Gerson LB, Shetler K, Triadafilopoulos G. Prevalence of Barrett's esophagus in asymptomatic individuals. Gastroenterology. 2002;123(2):461-7.
22. Cameron AJ. Epidemiology of Barrett's esophagus and adenocarcinoma. Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus / ISDE. 2002;15(2):106-8.
23. Heikkinen M, Pikkarainen P, Takala J, Rasanen H, Julkunen R. Etiology of dyspepsia: four hundred unselected consecutive patients in general practice. Scandinavian journal of gastroenterology. 1995;30(6):519-23.
24. Ronkainen J, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, et al. High prevalence of gastroesophageal reflux symptoms and esophagitis with or without symptoms in the general adult Swedish population: a Kalixanda study report. Scandinavian journal of gastroenterology. 2005;40(3):275-85.
25. Zagari RM, Pozzato P, Martuzzi C, Ricciardiello L, Lunedei V, Di Marco M, et al. 9 P Prevalence of asymptomatic endoscopic lesions of the upper gastrointestinal tract: Preliminary results of the Loiano-Monghidoro population study. Digestive and Liver Disease. 2002;34, Supplement 1(0):A112.
26. Kahrilas PJ, Shaheen NJ, Vaezi MF, Hiltz SW, Black E, Modlin IM, et al. American Gastroenterological Association Medical Position Statement on the management of gastroesophageal reflux disease. Gastroenterology. 2008;135(4):1383-91, 91 e1-5.

27. Gastroenterology BSo. Guidelines for the diagnosis and management of Barrett's columnar-lined oesophagus. 2005.
28. Katz PO, Gerson LB, Vela MF. Guidelines for the diagnosis and management of gastroesophageal reflux disease. The American journal of gastroenterology. 2013;108(3):308-28; quiz 29.
29. Dent J, El-Serag HB, Wallander MA, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut. 2005;54(5):710-7.
30. Revicki DA, Wood M, Maton PN, Sorensen S. The impact of gastroesophageal reflux disease on health-related quality of life. Am J Med. 1998;104(3):252-8.
31. Havelund T, Lind T, Wiklund I, Glise H, Hernqvist H, Lauritsen K, et al. Quality of life in patients with heartburn but without esophagitis: effects of treatment with omeprazole. The American journal of gastroenterology. 1999;94(7):1782-9.
32. Bjornsson E, Abrahamsson H, Simren M, Mattsson N, Jensen C, Agerforz P, et al. Discontinuation of proton pump inhibitors in patients on long-term therapy: a double-blind, placebo-controlled trial. Alimentary pharmacology & therapeutics. 2006;24(6):945-54.
33. Venables TL, Newland RD, Patel AC, Hole J, Copeman MB, Turbitt ML. Maintenance treatment for gastro-oesophageal reflux disease. A placebo-controlled evaluation of 10 milligrams omeprazole once daily in general practice. Scandinavian journal of gastroenterology. 1997;32(7):627-32.
34. Everhart JE, Ruhl CE. Burden of Digestive Diseases in the United States Part I: Overall and Upper Gastrointestinal Diseases. Gastroenterology. 2009;136(2):376-86.
35. Agreus L, Borgquist L. The cost of gastro-oesophageal reflux disease, dyspepsia and peptic ulcer disease in Sweden. PharmacoEconomics. 2002;20(5):347-55.
36. Jones R H HAPS, Philips J. Gastro-oesophageal reflux disease in primary care in Europe: Clinical presentation and endoscopic findings. European Journal of General Practice. 1995;1(4):149-54.
37. Mittal RK, Balaban DH. The esophagogastric junction. N Engl J Med. 1997;336(13):924-32.
38. Sharma P, Morales TG, Sampliner RE. Short segment Barrett's esophagus--the need for standardization of the definition and of endoscopic criteria. The American journal of gastroenterology. 1998;93(7):1033-6.
39. Wallner B. Endoscopically defined gastroesophageal junction coincides with the anatomical gastroesophageal junction. Surgical endoscopy. 2009;23(9):2155-8.
40. Kilgore SP, Ormsby AH, Gramlich TL, Rice TW, Richter JE, Falk GW, et al. The gastric cardia: fact or fiction? The American journal of gastroenterology. 2000;95(4):921-4.
41. Chandrasoma PT, Lokuhetty DM, Demeester TR, Bremmer CG, Peters JH, Oberg S, et al. Definition of histopathologic changes in gastroesophageal reflux disease. The American journal of surgical pathology. 2000;24(3):344-51.
42. Chandrasoma PT, Der R, Ma Y, Dalton P, Taira M. Histology of the gastroesophageal junction: an autopsy study. The American journal of surgical pathology. 2000;24(3):402-9.

43. Öberg S, Johansson J, Wenner J, Walther B. Metaplastic Columnar Mucosa in the Cervical Esophagus After Esophagectomy. Annals of surgery. 2002;235(3):338-45.
44. Oberg S, DeMeester TR, Peters JH, Hagen JA, Nigro JJ, DeMeester SR, et al. The extent of Barrett's esophagus depends on the status of the lower esophageal sphincter and the degree of esophageal acid exposure. The Journal Of Thoracic And Cardiovascular Surgery. 1999;117(3):572-80.
45. Spechler SJ, Goyal RK. Barrett's Esophagus. The New England journal of medicine. 1986;315(6):362-71.
46. Hamilton SR, Smith RR. The relationship between columnar epithelial dysplasia and invasive adenocarcinoma arising in Barrett's esophagus. American journal of clinical pathology. 1987;87(3):301-12.
47. Hirschowitz BI. A critical analysis, with appropriate controls, of gastric acid and pepsin secretion in clinical esophagitis. Gastroenterology. 1991;101(5):1149-58.
48. Fein M, Ritter MP, DeMeester TR, Oberg S, Peters JH, Hagen JA, et al. Role of the lower esophageal sphincter and hiatal hernia in the pathogenesis of gastroesophageal reflux disease. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 1999;3(4):405-10.
49. Penagini R, Mangano M, Bianchi PA. Effect of increasing the fat content but not the energy load of a meal on gastro-oesophageal reflux and lower oesophageal sphincter motor function. Gut. 1998;42(3):330-3.
50. Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J. Transient lower esophageal sphincter relaxation. Gastroenterology. 1995;109(2):601-10.
51. Sifrim D, Holloway R. Transient lower esophageal sphincter relaxations: how many or how harmful? The American journal of gastroenterology. 2001;96(9):2529-32.
52. Kahrilas PJ, Shi G, Manka M, Joehl RJ. Increased frequency of transient lower esophageal sphincter relaxation induced by gastric distention in reflux patients with hiatal hernia. Gastroenterology. 2000;118(4):688-95.
53. Trudgill NJ, Riley SA. Transient lower esophageal sphincter relaxations are no more frequent in patients with gastroesophageal reflux disease than in asymptomatic volunteers. The American journal of gastroenterology. 2001;96(9):2569-74.
54. Joelsson BE, DeMeester TR, Skinner DB, LaFontaine E, Waters PF, O'Sullivan GC. The role of the esophageal body in the antireflux mechanism. Surgery. 1982;92(2):417-24.
55. Orlando RC. Esophageal epithelial defenses against acid injury. The American journal of gastroenterology. 1994;89(8 Suppl):S48-52.
56. Castell DO, Murray JA, Tutuian R, Orlando RC, Arnold R. Review article: the pathophysiology of gastro-oesophageal reflux disease - oesophageal manifestations. Alimentary pharmacology & therapeutics. 2004;20 Suppl 9:14-25.
57. Kahrilas PJ, Dodds WJ, Hogan WJ, Kern M, Arndorfer RC, Reece A. Esophageal peristaltic dysfunction in peptic esophagitis. Gastroenterology. 1986;91(4):897-904.
58. Jones MP, Sloan SS, Rabine JC, Ebert CC, Huang CF, Kahrilas PJ. Hiatal hernia size is the dominant determinant of esophagitis presence and severity in gastroesophageal reflux disease. The American journal of gastroenterology. 2001;96(6):1711-7.

59. Ribolsi M, Balestrieri P, Emerenziani S, Guarino MP, Cicala M. Weak peristalsis with large breaks is associated with higher acid exposure and delayed reflux clearance in the supine position in GERD patients. The American journal of gastroenterology. 2014;109(1):46-51.
60. Helm JF, Dodds WJ, Hogan WJ, Soergel KH, Egide MS, Wood CM. Acid neutralizing capacity of human saliva. Gastroenterology. 1982;83(1 Pt 1):69-74.
61. Helm JF, Dodds WJ, Pelc LR, Palmer DW, Hogan WJ, Teeter BC. Effect of esophageal emptying and saliva on clearance of acid from the esophagus. N Engl J Med. 1984;310(5):284-8.
62. Helm JF, Dodds WJ, Hogan WJ. Salivary response to esophageal acid in normal subjects and patients with reflux esophagitis. Gastroenterology. 1987;93(6):1393-7.
63. Tobey NA, Carson JL, Alkiek RA, Orlando RC. Dilated intercellular spaces: a morphological feature of acid reflux--damaged human esophageal epithelium. Gastroenterology. 1996;111(5):1200-5.
64. Chiba N, De Gara CJ, Wilkinson JM, Hunt RH. Speed of healing and symptom relief in grade II to IV gastroesophageal reflux disease: A meta-analysis. Gastroenterology. 1997;112(6):1798-810.
65. Oberg S, Ritter MP, Crookes PF, Fein M, Mason RJ, Gadensytatter M, et al. Gastroesophageal reflux disease and mucosal injury with emphasis on short-segment Barrett's esophagus and duodenogastroesophageal reflux. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 1998;2(6):547-53; discussion 53-4.
66. Tobey NA, Hosseini SS, Caymaz-Bor C, Wyatt HR, Orlando GS, Orlando RC. The role of pepsin in acid injury to esophageal epithelium. The American journal of gastroenterology. 2001;96(11):3062-70.
67. Vaezi MF, Richter JE. Role of acid and duodenogastroesophageal reflux in gastroesophageal reflux disease. Gastroenterology. 1996;111(5):1192-9.
68. Beaumont H, Bennink RJ, de Jong J, Boeckxstaens GE. The position of the acid pocket as a major risk factor for acidic reflux in healthy subjects and patients with GORD. Gut. 2010;59(4):441-51.
69. Fletcher J, Wirz A, Young J, Vallance R, McColl KE. Unbuffered highly acidic gastric juice exists at the gastroesophageal junction after a meal. Gastroenterology. 2001;121(4):775-83.
70. Patti MG, Diener U, Tamburini A, Molena D, Way LW. Role of esophageal function tests in diagnosis of gastroesophageal reflux disease. Dig Dis Sci. 2001;46(3):597-602.
71. Fein M, Ireland AP, Ritter MP, Peters JH, Hagen JA, Bremner CG, et al. Duodenogastric reflux potentiates the injurious effects of gastroesophageal reflux. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 1997;1(1):27-32; discussion 3.
72. Kauer WK, Peters JH, DeMeester TR, Ireland AP, Bremner CG, Hagen JA. Mixed reflux of gastric and duodenal juices is more harmful to the esophagus than gastric juice alone. The need for surgical therapy re-emphasized. Annals of surgery. 1995;222(4):525-31; discussion 31-3.

73. Richter JE. Current Diagnosis and Management of Suspected Reflux Symptoms Refractory to Proton Pump Inhibitor Therapy. Gastroenterology & Hepatology. 2014;10(9):547-55.
74. Sifrim D, Zerbib F. Diagnosis and management of patients with reflux symptoms refractory to proton pump inhibitors. Gut. 2012;61(9):1340-54.
75. Lundell LR, Dent J, Bennett JR, Blum AL, Armstrong D, Galmiche JP, et al. Endoscopic assessment of oesophagitis: clinical and functional correlates and further validation of the Los Angeles classification. Gut. 1999;45(2):172-80.
76. Pisegna J, Holtmann G, Howden CW, Katelaris PH, Sharma P, Spechler S, et al. Review article: oesophageal complications and consequences of persistent gastro-oesophageal reflux disease. Alimentary pharmacology & therapeutics. 2004;20 Suppl 9:47-56.
77. Rantanen TK, Sihvo EI, Rasanen JV, Salo JA. Gastroesophageal reflux disease as a cause of death is increasing: analysis of fatal cases after medical and surgical treatment. The American journal of gastroenterology. 2007;102(2):246-53.
78. Bytzer P, Christensen PB, Damkier P, Vinding K, Seersholm N. Adenocarcinoma of the esophagus and Barrett's esophagus: a population-based study. The American journal of gastroenterology. 1999;94(1):86-91.
79. Blot Wj DSSFJF, Jr. Continuing climb in rates of esophageal adenocarcinoma: An update. JAMA. 1993;270(11):1320-.
80. Pera M, Cameron AJ, Trastek VF, Carpenter HA, Zinsmeister AR. Increasing incidence of adenocarcinoma of the esophagus and esophagogastric junction. Gastroenterology. 1993;104(2):510-3.
81. Stein HJ, Siewert JR. Barrett's esophagus: pathogenesis, epidemiology, functional abnormalities, malignant degeneration, and surgical management. Dysphagia. 1993;8(3):276-88.
82. Winters C, Jr., Spurling TJ, Chobanian SJ, Curtis DJ, Esposito RL, Hacker JF, 3rd, et al. Barrett's esophagus. A prevalent, occult complication of gastroesophageal reflux disease. Gastroenterology. 1987;92(1):118-24.
83. Skinner DB, Walther BC, Riddell RH, Schmidt H, Iascone C, DeMeester TR. Barrett's esophagus. Comparison of benign and malignant cases. Annals of surgery. 1983;198(4):554-65.
84. Koop H. Reflux disease and Barrett's esophagus. Endoscopy. 2000;32(2):101-7.
85. Spechler SJ, Zeroogian JM, Antonioli DA, Wang HH, Goyal RK. Prevalence of metaplasia at the gastro-oesophageal junction. The Lancet. 1994;344(8936):1533-6.
86. Mann NS, Tsai MF, Nair PK. Barrett's esophagus in patients with symptomatic reflux esophagitis. The American journal of gastroenterology. 1989;84(12):1494-6.
87. Hansson LE, Sparén P, Nyrén O. Increasing incidence of both major histological types of esophageal carcinomas among men in Sweden. International journal of cancer. 1993;54(3):402-7.
88. Shaheen NJ, Crosby MA, Bozymski EM, Sandler RS. Is there publication bias in the reporting of cancer risk in Barrett's esophagus? Gastroenterology. 2000;119(2):333-8.

89. Lerut T, De Leyn P, Coosemans W, Van Raemdonck D, Scheys I, LeSaffre E. Surgical strategies in esophageal carcinoma with emphasis on radical lymphadenectomy. Annals of surgery. 1992;216(5):583-90.
90. Rudolph RE, Vaughan TL, Storer BE, Haggitt RC, Rabinovitch PS, Levine DS, et al. Effect of segment length on risk for neoplastic progression in patients with Barrett esophagus. Ann Intern Med. 2000;132(8):612-20.
91. Spechler S. J. The Columnar lined Esophagus. Gastroenterology clinics of North America. 1997;26(3): 455-701.
92. Bartlett DW, Evans DF, Anggiansah A, Smith BG. A study of the association between gastro-oesophageal reflux and palatal dental erosion. British dental journal. 1996;181(4):125-31.
93. Moazzez R, Bartlett D, Anggiansah A. Dental erosion, gastro-oesophageal reflux disease and saliva: how are they related? Journal of dentistry. 2004;32(6):489-94.
94. Ford CN. Evaluation and management of laryngopharyngeal reflux. Jama. 2005;294(12):1534-40.
95. Jacobson BC, Somers SC, Fuchs CS, Kelly CP, Camargo CA, Jr. Body-mass index and symptoms of gastroesophageal reflux in women. N Engl J Med. 2006;354(22):2340-8.
96. Fraser-Moodie CA, Norton B, Gornall C, Magnago S, Weale AR, Holmes GK. Weight loss has an independent beneficial effect on symptoms of gastro-oesophageal reflux in patients who are overweight. Scandinavian journal of gastroenterology. 1999;34(4):337-40.
97. Mathus-Vliegen EM, Tygat GN. Gastro-oesophageal reflux in obese subjects: influence of overweight, weight loss and chronic gastric balloon distension. Scandinavian journal of gastroenterology. 2002;37(11):1246-52.
98. Pandolfino JE, Kahrilas PJ. Smoking and gastro-oesophageal reflux disease. European journal of gastroenterology & hepatology. 2000;12(8):837-42.
99. Sandmark S, Carlsson R, Fausa O, Lundell L. Omeprazole or ranitidine in the treatment of reflux esophagitis. Results of a double-blind, randomized, Scandinavian multicenter study. Scandinavian journal of gastroenterology. 1988;23(5):625-32.
100. Carlsson R, Dent J, Watts R, Riley S, Sheikh R, Hatlebakk J, et al. Gastro-oesophageal reflux disease in primary care: an international study of different treatment strategies with omeprazole. International GORD Study Group. European journal of gastroenterology & hepatology. 1998;10(2):119-24.
101. Dean BB, Gano AD, Jr., Knight K, Ofman JJ, Fass R. Effectiveness of proton pump inhibitors in nonerosive reflux disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2004;2(8):656-64.
102. Talley NJ, Armstrong D, Junghard O, Wiklund I. Predictors of treatment response in patients with non-erosive reflux disease. Alimentary pharmacology & therapeutics. 2006;24(2):371-6.
103. Jackson PG, Gleiber MA, Askari R, Evans SR. Predictors of outcome in 100 consecutive laparoscopic antireflux procedures. American journal of surgery. 2001;181(3):231-5.

104. O'Boyle CJ, Watson DI, Jamieson GG, Myers JC, Game PA, Devitt PG. Division of short gastric vessels at laparoscopic nissen fundoplication: a prospective double-blind randomized trial with 5-year follow-up. Annals of surgery. 2002;235(2):165-70.
105. Rantanen TK, Salo JA, Sipponen JT. Fatal and life-threatening complications in antireflux surgery: analysis of 5,502 operations. The British journal of surgery. 1999;86(12):1573-7.
106. Dallemagne B, Perretta S. Twenty years of laparoscopic fundoplication for GERD. World journal of surgery. 2011;35(7):1428-35.
107. Frazzoni M, Piccoli M, Conigliaro R, Frazzoni L, Melotti G. Laparoscopic fundoplication for gastroesophageal reflux disease. World journal of gastroenterology : WJG. 2014;20(39):14272-9.
108. Watson TJ, Peters JH. Lower esophageal sphincter injections for the treatment of gastroesophageal reflux disease. Thoracic surgery clinics. 2005;15(3):405-15.
109. Schwartz MP, Smout AJ. Review article: The endoscopic treatment of gastro-oesophageal reflux disease. Alimentary pharmacology & therapeutics. 2007;26 Suppl 2:1-6.
110. DeVault KR, Castell DO. Updated guidelines for the diagnosis and treatment of gastroesophageal reflux disease. The American journal of gastroenterology. 2005;100(1):190-200.
111. Venables TL, Newland RD, Patel AC, Hole J, Wilcock C, Turbitt ML. Omeprazole 10 milligrams once daily, omeprazole 20 milligrams once daily, or ranitidine 150 milligrams twice daily, evaluated as initial therapy for the relief of symptoms of gastro-oesophageal reflux disease in general practice. Scandinavian journal of gastroenterology. 1997;32(10):965-73.
112. Zaninotto G, DeMeester TR, Schwizer W, Johansson KE, Cheng SC. The lower esophageal sphincter in health and disease. American journal of surgery. 1988;155(1):104-11.
113. Levine MS, Rubesin SE. History and Evolution of the Barium Swallow for Evaluation of the Pharynx and Esophagus. Dysphagia. 2017;32(1):55-72.
114. Lin OS, Schembre DB, Mergener K, Spaulding W, Lomah N, Ayub K, et al. Blinded comparison of esophageal capsule endoscopy versus conventional endoscopy for a diagnosis of Barrett's esophagus in patients with chronic gastroesophageal reflux. Gastrointestinal endoscopy. 2007;65(4):577-83.
115. Sharma P, Wani S, Rastogi A, Bansal A, Higbee A, Mathur S, et al. The diagnostic accuracy of esophageal capsule endoscopy in patients with gastroesophageal reflux disease and Barrett's esophagus: a blinded, prospective study. The American journal of gastroenterology. 2008;103(3):525-32.
116. Stipa F, Stein HJ, Feussner H, Kraemer S, Siewert JR. Assessment of non-acid esophageal reflux: comparison between long-term reflux aspiration test and fiberoptic bilirubin monitoring. Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus / ISDE. 1997;10(1):24-8.
117. Okholm M, Sorensen H, Wallin L, Boesby S. Bile reflux into the esophagus. Bilitec 2000 measurements in normal subjects and in patients after Nissen fundoplication. Scandinavian journal of gastroenterology. 1999;34(7):653-7.

118. Pace F, Sangaletti O, Pallotta S, Molteni P, Porro GB. Biliary reflux and non-acid reflux are two distinct phenomena: a comparison between 24-hour multichannel intraesophageal impedance and bilirubin monitoring. Scandinavian journal of gastroenterology. 2007;42(9):1031-9.
119. Herbella FA. Critical analysis of esophageal multichannel intraluminal impedance monitoring 20 years later. ISRN gastroenterology. 2012;2012:903240.
120. Bredenoord AJ, Draaisma WA, Weusten BL, Gooszen HG, Smout AJ. Mechanisms of acid, weakly acidic and gas reflux after anti-reflux surgery. Gut. 2008;57(2):161-6.
121. Francis DO, Goutte M, Slaughter JC, Garrett CG, Hagaman D, Holzman MD, et al. Traditional reflux parameters and not impedance monitoring predict outcome after fundoplication in extraesophageal reflux. The Laryngoscope. 2011;121(9):1902-9.
122. Johnsson F, Hatlebakk JG, Klintenberg AC, Roman J, Toth E, Stubberod A, et al. One-week esomeprazole treatment: an effective confirmatory test in patients with suspected gastroesophageal reflux disease. Scandinavian journal of gastroenterology. 2003;38(4):354-9.
123. Schenk BE, Kuipers EJ, Klinkenberg-Knol EC, Festen HP, Jansen EH, Tuynman HA, et al. Omeprazole as a diagnostic tool in gastroesophageal reflux disease. The American journal of gastroenterology. 1997;92(11):1997-2000.
124. Wang WH, Huang JQ, Zheng GF, Wong WM, Lam SK, Karlberg J, et al. Is proton pump inhibitor testing an effective approach to diagnose gastroesophageal reflux disease in patients with noncardiac chest pain?: a meta-analysis. Arch Intern Med. 2005;165(11):1222-8.
125. Campos GM, Peters JH, DeMeester TR, Oberg S, Crookes PF, Tan S, et al. Multivariate analysis of factors predicting outcome after laparoscopic Nissen fundoplication. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 1999;3(3):292-300.
126. Herbella FA, Nipominick I, Patti MG. From sponges to capsules. The history of esophageal pH monitoring. Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus / ISDE. 2009;22(2):99-103.
127. Johnson LF, Demeester TR. Twenty-four-hour pH monitoring of the distal esophagus. A quantitative measure of gastroesophageal reflux. The American journal of gastroenterology. 1974;62(4):325-32.
128. Spencer J. Prolonged pH recording in the study of gastro-oesophageal reflux. The British journal of surgery. 1969;56(12):912-4.
129. Opekun AR, Smith JL, Graham DY. Antimony electrodes. Mucosal potential differences and buffer composition adversely affect pH measurements in the stomach. Dig Dis Sci. 1990;35(8):950-5.
130. Geus WP, Smout AJ, Kooiman JC, Lamers CB, Gues JW. Glass and antimony electrodes for long-term pH monitoring: a dynamic in vitro comparison. European journal of gastroenterology & hepatology. 1995;7(1):29-35.
131. Hemmink GJ, Weusten BL, Oors J, Bredenoord AJ, Timmer R, Smout AJ. Ambulatory oesophageal pH monitoring: a comparison between antimony, ISFET, and glass pH electrodes. European journal of gastroenterology & hepatology. 2010;22(5):572-7.

132. Pandolfino JE, Ghosh S, Zhang Q, Heath M, Bombeck T, Kahrilas PJ. Slimline vs. glass pH electrodes: what degree of accuracy should we expect? Alimentary pharmacology & therapeutics. 2006;23(2):331-40.
133. Anggiansah A, Sumboonnanonda K, Wang J, Linsell J, Hale P, Owen WJ. Significantly reduced acid detection at 10 centimeters compared to 5 centimeters above lower esophageal sphincter in patients with acid reflux. The American journal of gastroenterology. 1993;88(6):842-6.
134. Wenner J, Johnsson F, Johansson J, Oberg S. Acid reflux immediately above the squamocolumnar junction and in the distal esophagus: simultaneous pH monitoring using the wireless capsule pH system. The American journal of gastroenterology. 2006;101(8):1734-41.
135. Fletcher J, Wirz A, Henry E, McColl KE. Studies of acid exposure immediately above the gastro-oesophageal squamocolumnar junction: evidence of short segment reflux. Gut. 2004;53(2):168-73.
136. Wenner J, Johnsson F, Johansson J, Öberg S. Wireless oesophageal pH monitoring: Feasibility, safety and normal values in healthy subjects. Scandinavian Journal of Gastroenterology. 2005;40(7):768-74.
137. Lee YC, Wang HP, Chiu HM, Huang SP, Tu CH, Wu MS, et al. Patients with functional heartburn are more likely to report retrosternal discomfort during wireless pH monitoring. Gastrointestinal endoscopy. 2005;62(6):834-41.
138. Pandolfino JE, Richter JE, Ours T, Guardino JM, Chapman J, Kahrilas PJ. Ambulatory esophageal pH monitoring using a wireless system. The American journal of gastroenterology. 2003;98(4):740-9.
139. des Varannes SB, Mion F, Ducrotte P, Zerbib F, Denis P, Ponchon T, et al. Simultaneous recordings of oesophageal acid exposure with conventional pH monitoring and a wireless system (Bravo). Gut. 2005;54(12):1682-6.
140. Bhat YM, McGrath KM, Bielefeldt K. Wireless esophageal pH monitoring: new technique means new questions. J Clin Gastroenterol. 2006;40(2):116-21.
141. Wenner J, Johansson J, Johnsson F, Oberg S. Optimal thresholds and discriminatory power of 48-h wireless esophageal pH monitoring in the diagnosisof GERD. The American journal of gastroenterology. 2007;102(9):1862-9.
142. Ayazi S, Lipham JC, Portale G, Peyre CG, Streets CG, Leers JM, et al. Bravo catheter-free pH monitoring: normal values, concordance, optimal diagnostic thresholds, and accuracy. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2009;7(1):60-7.
143. Johnsson F, Joelsson B, Isberg PE. Ambulatory 24 hour intraesophageal pH-monitoring in the diagnosis of gastroesophageal reflux disease. Gut. 1987;28(9):1145-50.
144. Richter JE, Bradley LA, DeMeester TR, Wu WC. Normal 24-hr ambulatory esophageal pH values. Influence of study center, pH electrode, age, and gender. Dig Dis Sci. 1992;37(6):849-56.
145. Kahrilas PJ, Quigley EM. Clinical esophageal pH recording: a technical review for practice guideline development. Gastroenterology. 1996;110(6):1982-96.

146. Jamieson JR, Stein HJ, DeMeester TR, Bonavina L, Schwizer W, Hinder RA, et al. Ambulatory 24-h esophageal pH monitoring: normal values, optimal thresholds, specificity, sensitivity, and reproducibility. The American journal of gastroenterology. 1992;87(9):1102-11.
147. Hirano I, Richter JE. ACG practice guidelines: esophageal reflux testing. The American journal of gastroenterology. 2007;102(3):668-85.
148. Bremner RM, Bremner CG, DeMeester TR. Gastroesophageal reflux: the use of pH monitoring. Current problems in surgery. 1995;32(6):429-558.
149. Tuttle SG, Rufin F, Bettarello A. The physiology of heartburn. Ann Intern Med. 1961;55:292-300.
150. Goldberg HI, Dodds WJ, Gee S, Montgomery C, Zboralske FF. Role of acid and pepsin in acute experimental esophagitis. Gastroenterology. 1969;56(2):223-30.
151. Piper DW, Fenton BH. pH stability and activity curves of pepsin with special reference to their clinical importance. Gut. 1965;6(5):506-8.
152. Schindlbeck NE, Heinrich C, Konig A, Dendorfer A, Pace F, Muller-Lissner SA. Optimal thresholds, sensitivity, and specificity of long-term pH-metry for the detection of gastroesophageal reflux disease. Gastroenterology. 1987;93(1):85-90.
153. Bredenoord AJ, Weusten BL, Smout AJ. Symptom association analysis in ambulatory gastro-oesophageal reflux monitoring. Gut. 2005;54(12):1810-7.
154. Lam HG, Breumelhof R, Roelofs JM, Van Berge Henegouwen GP, Smout AJ. What is the optimal time window in symptom analysis of 24-hour esophageal pressure and pH data? Dig Dis Sci. 1994;39(2):402-9.
155. Wiener GJ, Richter JE, Copper JB, Wu WC, Castell DO. The symptom index: a clinically important parameter of ambulatory 24-hour esophageal pH monitoring. Am J Gastroenterol. 1988;83(4):358-61.
156. Breumelhof R, Smout AJ. The symptom sensitivity index: a valuable additional parameter in 24-hour esophageal pH recording. Am J Gastroenterol. 1991;86(2):160-4.
157. Weusten BL, Roelofs JM, Akkermans LM, Van Berge-Henegouwen GP, Smout AJ. The symptom-association probability: an improved method for symptom analysis of 24-hour esophageal pH data. Gastroenterology. 1994;107(6):1741-5.
158. Fass R, Hell R, Sampliner RE, Pulliam G, Graver E, Hartz V, et al. Effect of ambulatory 24-hour esophageal pH monitoring on reflux-provoking activities. Dig Dis Sci. 1999;44(11):2263-9.
159. Pandolfino JE, Bianchi LK, Lee TJ, Hirano I, Kahrilas PJ. Esophagogastric junction morphology predicts susceptibility to exercise-induced reflux. The American journal of gastroenterology. 2004;99(8):1430-6.
160. Wenner J, Johnsson F, Johansson J, Oberg S. Wireless esophageal pH monitoring is better tolerated than the catheter-based technique: results from a randomized cross-over trial. The American journal of gastroenterology. 2007;102(2):239-45.
161. Tseng D, Rizvi AZ, Fennerty MB, Jobe BA, Diggs BS, Sheppard BC, et al. Forty-eight-hour pH monitoring increases sensitivity in detecting abnormal esophageal acid

exposure. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2005;9(8):1043-51; discussion 51-2.

162. Mattioli S, Pilotti V, Spangaro M, Grigioni WF, Zannoli R, Felice V, et al. Reliability of 24-hour home esophageal pH monitoring in diagnosis of gastroesophageal reflux. Dig Dis Sci. 1989;34(1):71-8.
163. Kahrilas PJ. Gastroesophageal reflux disease. Jama. 1996;276(12):983-8.
164. Bredenoord AJ, Weusten BL, Curvers WL, Timmer R, Smout AJ. Determinants of perception of heartburn and regurgitation. Gut. 2006;55(3):313-8.
165. Hall MG, Wenner J, Oberg S. The combination of pH monitoring in the most distal esophagus and symptom association analysis markedly improves the clinical value of esophageal pH tests. Scandinavian journal of gastroenterology. 2016;51(2):129-36.
166. Wallner B, Sylvan A, Stenling R, Janunger KG. The Esophageal Z-Line Appearance Correlates to the Prevalence of Intestinal Metaplasia. Scandinavian Journal of Gastroenterology. 2000;35(1):17-22.
167. Csendes A, Maluenda F, Braghetto I, Csendes P, Henriquez A, Quesada MS. Location of the lower oesophageal sphincter and the squamous columnar mucosal junction in 109 healthy controls and 778 patients with different degrees of endoscopic oesophagitis. Gut. 1993;34(1):21-7.
168. Metz CE. Basic principles of ROC analysis. Seminars in nuclear medicine. 1978;8(4):283-98.
169. Zweig MH. ROC plots display test accuracy, but are still limited by the study design. Clinical chemistry. 1993;39(6):1345-6.
170. Greiner M, Pfeiffer D, Smith RD. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Preventive veterinary medicine. 2000;45(1-2):23-41.
171. Galmiche JP, Clouse RE, Balint A, Cook IJ, Kahrilas PJ, Paterson WG, et al. Functional esophageal disorders. Gastroenterology. 2006;130(5):1459-65.
172. Emde C, Cilluffo T, Bauerfeind P, Blum AL. Combined esophageal and gastric pH-metry in healthy volunteers. Influence of cable through LES and effect of misoprostol. Dig Dis Sci. 1989;34(1):79-82.
173. Aanen MC, Bredenoord AJ, Numans ME, Samson M, Smout AJ. Reproducibility of symptom association analysis in ambulatory reflux monitoring. The American journal of gastroenterology. 2008;103(9):2200-8.
174. Taghavi SA, Ghasedi M, Saberi-Firoozi M, Alizadeh-Naeeni M, Bagheri-Lankarani K, Kaviani MJ, et al. Symptom association probability and symptom sensitivity index: preferable but still suboptimal predictors of response to high dose omeprazole. Gut. 2005;54(8):1067-71.
175. Clarke AT, Wirz AA, Manning JJ, Ballantyne SA, Alcorn DJ, McColl KE. Severe reflux disease is associated with an enlarged unbuffered proximal gastric acid pocket. Gut. 2008;57(3):292-7.
176. Kuiken S, Van Den Elzen B, Tytgat G, Bennink R, Boeckxstaens G. Evidence for pooling of gastric secretions in the proximal stomach in humans using single photon computed tomography. Gastroenterology. 2002;123(6):2157-8; author reply 8.

177. Clarke AT, Wirz AA, Seenan JP, Manning JJ, Gillen D, McColl KE. Paradox of gastric cardia: it becomes more acidic following meals while the rest of stomach becomes less acidic. Gut. 2009;58(7):904-9.
178. Simonian HP, Vo L, Doma S, Fisher RS, Parkman HP. Regional postprandial differences in pH within the stomach and gastroesophageal junction. Dig Dis Sci. 2005;50(12):2276-85.
179. Pandolfino JE, Zhang Q, Ghosh SK, Post J, Kwiatek M, Kahrilas PJ. Acidity surrounding the squamocolumnar junction in GERD patients: "acid pocket" versus "acid film". The American journal of gastroenterology. 2007;102(12):2633-41.
180. Hila A, Bouali H, Xue S, Knuff D, Castell DO. Postprandial stomach contents have multiple acid layers. J Clin Gastroenterol. 2006;40(7):612-7.
181. Berne RM, Koeppen BM, Stanton BA. Berne & Levy physiology. Philadelphia, PA: Mosby/Elsevier; 2010.
182. Pandolfino JE, Schreiner MA, Lee TJ, Zhang Q, Kahrilas PJ. Bravo capsule placement in the gastric cardia: a novel method for analysis of proximal stomach acid environment. The American journal of gastroenterology. 2005;100(8):1721-7.
183. Hirota WK, Loughney TM, Lazas DJ, Maydonovitch CL, Rholl V, Wong RK. Specialized intestinal metaplasia, dysplasia, and cancer of the esophagus and esophagogastric junction: prevalence and clinical data. Gastroenterology. 1999;116(2):277-85.
184. Clémençon G, Gloor F. Endoscopic and histological changes at the Z-line (author's transl). Deutsche medizinische Wochenschrift. 1977;102(16):609-12.
185. Savary M, Miller G. The esophagus: handbook and atlas of endoscopy. Solothurn, Switzerland: Gassmann ; Westbury, N. Y. : distributed in the U. S. by TAG Photographic; 1978. 248 p. p.
186. DeNardi FGR, R.B. The Normal Esophagus. The American Journal of Surgical Pathology. 1991;15(3):296-309.
187. Wallner B, Sylvan A, Stenling R, Janunger KG. The Z-line appearance and prevalence of intestinal metaplasia among patients without symptoms or endoscopical signs indicating gastroesophageal reflux. Surg Endosc. 2001;15(8):886-9.
188. Oberg S, Peters JH, DeMeester TR, Chandrasoma P, Hagen JA, Ireland AP, et al. Inflammation and specialized intestinal metaplasia of cardiac mucosa is a manifestation of gastroesophageal reflux disease. Annals of surgery. 1997;226(4):522-30; discussion 30-2.
189. Voutilainen M, Sipponen P, Mecklin JP, Juhola M, Farkkila M. Gastroesophageal reflux disease: prevalence, clinical, endoscopic and histopathological findings in 1,128 consecutive patients referred for endoscopy due to dyspeptic and reflux symptoms. Digestion. 2000;61(1):6-13.
190. Oksanen A, Sipponen P, Karttunen R, Rautelin H. Inflammation and intestinal metaplasia at the squamocolumnar junction in young patients with or without Helicobacter pylori infection. Gut. 2003;52(2):194-8.
191. Carton E, Mulligan ED, Keeling PW, Tanner A, McDonald G, Reynolds JV. Specialized intestinal metaplasia: analysis of prevalence, risk factors and association

with gastro-oesophageal reflux disease. The British journal of surgery. 2000;87(3):362-73.

192. Goldblum JR, Vicari JJ, Falk GW, Rice TW, Peek RM, Easley K, et al. Inflammation and intestinal metaplasia of the gastric cardia: the role of gastroesophageal reflux and H. pylori infection. Gastroenterology. 1998;114(4):633-9.
193. Morales TG, Sampliner RE, Bhattacharyya A. Intestinal metaplasia of the gastric cardia. The American journal of gastroenterology. 1997;92(3):414-8.
194. Morini S, Zullo A, Hassan C, Lorenzetti R, Stella F, Martini MT. Gastric cardia inflammation: role of Helicobacter pylori infection and symptoms of gastroesophageal reflux disease. The American journal of gastroenterology. 2001;96(8):2337-40.
195. A Hackelsberger TGn, V Schultze, G Manes, J-E Dominguez-Muñoz, A Roessner, P Malfertheiner. Intestinal metaplasia at the gastro-oesophageal junction: Helicobacter pylori gastritis or gastro-oesophageal reflux disease? Gut 1998;43:17-21.
196. Pascarenco OD, Boeriu A, Mocan S, Pascarenco G, Drasoveanu S, Galeanu M, et al. Barrett's esophagus and intestinal metaplasia of gastric cardia: prevalence, clinical, endoscopic and histological features. Journal of gastrointestinal and liver diseases : JGLD. 2014;23(1):19-25.
197. Aksglaede K, Funch-Jensen P, Thommesen P. Intra-esophageal pH probe movement during eating and talking. A videoradiographic study. Acta radiologica (Stockholm, Sweden : 1987). 2003;44(2):131-5.
198. Kahrilas PJ, Wu S, Lin S, Pouderoux P. Attenuation of esophageal shortening during peristalsis with hiatus hernia. Gastroenterology. 1995;109(6):1818-25.
199. Law S, Lam KY, Chu KM, Wong J. Specialized intestinal metaplasia and carditis at the gastroesophageal junction in Chinese patients undergoing endoscopy. The American journal of gastroenterology. 2002;97(8):1924-9.
200. Guelrud M, Herrera I, Essenfeld H, Castro J, Antonioli DA. Intestinal metaplasia of the gastric cardia: A prospective study with enhanced magnification endoscopy. The American journal of gastroenterology. 2002;97(3):584-9.
201. Sharma P, McElhinney C, Topalovski M, Mayo MS, McGregor DH, Weston A. Detection of cardia intestinal metaplasia: do the biopsy number and location matter? The American journal of gastroenterology. 2004;99(12):2424-8.
202. Wolf C, Seldenrijk CA, Timmer R, Breumelhof R, Smout AJ. Epidemiological, clinical, and histological characteristics of intestinal metaplasia at the oesophagogastric junction compared to short segments of columnar-lined oesophagus. European journal of gastroenterology & hepatology. 2001;13(2):113-20.
203. Odze RD. Pathology of the gastroesophageal junction. Seminars in diagnostic pathology. 2005;22(4):256-65.
204. Sharma P, Morales TG, Bhattacharyya A, Garewal HS, Sampliner RE. Dysplasia in short-segment Barrett's esophagus: a prospective 3-year follow-up. The American journal of gastroenterology. 1997;92(11):2012-6.