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ASPECTS ON ENDOSCOPIC CHARACTERIZATION AND CLINICAL MANAGEMENT OF BARRETT'S ESOPHAGUS

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ASPECTS ON ENDOSCOPIC CHARACTERIZATION AND CLINICAL MANAGEMENT OF BARRETT'S ESOPHAGUS

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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William James describes a man who got the experience from laughing-gas; whenever he was under its influence, he knew the secret of the universe, but when he came to, he had forgotten it. At last, with immense effort, he wrote down the secret before the vision had faded. When completely recovered, he rushed to see what he had written. It was ‘A smell of petroleum prevails throughout’.

Bertrand Russell
A History of Western Philosophy

To Matias, Francisca, Pia and Raquel

ABSTRACT

Barrett's esophagus (BE) is considered to result from prolonged gastroesophageal reflux and is the only known precursor of esophageal adenocarcinoma.
The clinical management of BE patients aims to control esophageal reflux to reduce mucosal injury and neoplastic progression, and to detect early neoplastic lesions in Barrett's mucosa, suitable for curative endoscopic treatment.

The first part of this thesis evaluates the effect of a stepwise increase in the dose of proton pump inhibitors (PPI), on esophageal acidic reflux, symptoms and histology in long segment BE patients (group 1, n=24). We also compare these outcomes in BE patients under PPI with the results of BE patients after clinically successful fundoplication (group 2, n=30). In all but one patient in group 1, it was possible to normalize acid reflux with PPI, resulting in improvements in symptom scores. However, symptomatic amelioration was only significant in the first step of PPI treatment. Patients with PPI or fundoplication had the same levels of symptom scores. Normalization of the acid reflux in both groups was associated with reductions of papillary length, thickness of the basal cell layers, dilation of intercellular spaces, and acute and chronic inflammation of the squamous epithelium. We did not find a significant change in markers of proliferation and differentiation in Barrett's mucosa associated with normalization of acid reflux in either group.

The second part of this thesis assesses 3 different endoscopic classification systems, Amsterdam, Kansas and Nottingham, developed for the characterization of Barrett's mucosa. These classifications use magnification endoscopy with narrow band imaging (ME-NBI) for the identification of intestinal metaplasia and dysplasia in Barrett's mucosa. We used 84 video segments from Barrett's mucosa, that were randomly selected and blindly evaluated by 9 observers with different expertise in the field. All classifications were feasibly but showed suboptimal accuracy and low inter-observer agreement, with slightly better results for the Amsterdam classification.

The last part of this thesis evaluates the role of a structured learning program for the application of the Amsterdam classification system. We used the first 70 videos from the 84 randomly selected videos from the previous study. While, during the learning process, there was a decrease in the time spent for evaluation and an increase in declared certainty of prediction, the accuracy in histological prediction did not improve. This classification system was found to be suboptimal in terms of accuracy and inter- and intra-observer agreements.

This thesis shows that, in long segment BE patients, acid reflux and symptom scores correlated through several steps of the PPI treatment process, achieving the same level as after a successful fundoplication. If a single dose of PPI is associated with marked improvement of symptoms, higher doses still may be needed for complete acid suppression. Minor changes were found among morphological markers of reflux disease, both in the glandular and in the squamous epithelium, irrespective of medical or surgical treatment. Our results underscore the questionable utility of ME-NBI classification systems for clinical routine practice in BE.

Keywords: Barrett's esophagus, anti-reflux surgery, proton pump inhibitors, narrow band imaging.

LIST OF SCIENTIFIC PAPERS

I. Francisco Baldaque-Silva, Michael Vieth, Mumen Debel, Bengt Håkanson, Anders Thorell, Nuno Lunet, Huan Song, Miguel Mascarenhas-Saraiva, Gisela Pereira, Lars Lundell, Hanns-Ulrich Marschall.
Impact of gastroesophageal reflux control through tailored proton pump inhibition therapy or fundoplication in patients with Barrett's esophagus.
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Gastrointestinal Endoscopy 2011 Jan;73(1):7-14

III. Francisco Baldaque-Silva, Margarida Marques, Nuno Lunet, Gonçalo Themudo, Kenichi Goda, Ervin Toth, José Soares, Pedro Bastos, Rosa Ramalho, Pedro Pereira, Miguel Coimbra, Michael Vieth, Mário Dinis-Ribeiro, Guilherme Macedo, Lars Lundell, Hanns-Ulrich Marschall. **Endoscopic assessment and grading of Barrett's esophagus using magnification endoscopy and narrow band imaging: impact of structured learning and experience on the accuracy of the Amsterdam classification system.**
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LIST OF ABBREVIATIONS

ASA	Acetylsalicyclic acid
ARS	Anti-reflux surgery
BE	Barrett's esophagus
EAC	Esophageal adenocarcinoma
GERD	Gastroesophageal reflux disease
GEJ	Gastroesophageal junction
GI	Gastrointestinal
H&E	Hematoxylin and eosin
HGD	High-grade dysplasia
HRQL	Health related quality of live
H2RA	Histamine-2 receptor antagonists
IHC	Immunohistochemistry
IM	Intestinal metaplasia
LES	Lower esophageal sphincter
LGD	Low-grade dysplasia
LR	Likelihood ratios
NBI	Narrow band imaging
NSAID	Nonsteroidal anti-inflammatory drug
PPI	Proton pump inhibitors
RCT	Randomized control trial

1 INTRODUCTION

Barrett's esophagus (BE) is a condition where the squamous esophageal epithelium is replaced by metaplastic columnar epithelium. This novel epithelium is considered to carry malignant potential and harbor a risk for esophageal adenocarcinoma, one of the cancers with highest increase in incidence in the Western World. Our understanding of BE has improved over time, but this process has been hampered by evolving definitions, lack of global consensus on diagnostic criteria, and the array of symptoms and clinical progression observed in patients with BE. Advances had been registered in endoscopic detection and characterization on BE, not only due to improvements in detection tools, but also due to higher awareness among clinicians and endoscopists of this condition. These advances have been moving along with a dramatic change in the management and treatment of patients with BE. The previous reality of late diagnostic, random follow-up, palliative or surgical treatment and low survival of neoplastic BE, has been steadily replaced by earlier diagnosis, improved endoscopic characterization, standardized follow-up, and pharmacological and endoscopic treatment with increased survival.

1.1 BARRETT'S ESOPHAGUS-HISTORICAL REMARKS

The definition of Barrett's esophagus has been controversial since it was coined by Allison and Johnstone in 1953, in reference to the famous Australian surgeon Norman Barrett (1, 2). For a better understanding of these controversies, it is important to appraise some landmarks in BE history. The German pathologist Albers reported the presence of peptic ulcer in the esophagus in 1839 (3), but Tileston was the first to describe the columnar lined esophagus associated with esophageal ulcerations in 1906, relating it to an insufficient cardia (4). Norman Barrett defined the esophagus by the presence of squamous epithelium in 1950, proposing that those previously described ulcerations were in fact ulcerations in an intrathoracic tubular stomach due a congenital short esophagus (3). These assumptions were made due to the fact that the columnar epithelium found adjacent to the ulcers was histologically of gastric-columnar type. Allison and Johnstone contested this concept, since the columnar epithelium could harbor squamous epithelium islands, and like in the esophagus, there were submucosal glands, the muscularis propria resembled the typical esophagus and there was no peritoneal covering of the organ. Barrett accepted this reasoning in 1957, proposing the definition of this condition as "lower oesophagus lined by columnar epithelium" (5). In 1961, Hayward defended that the distal 1-2 cm of the esophagus was normally covered by gastric junctional type mucosa that worked as a buffer zone between acid producing gastric mucosa and squamous epithelium (6). This non- founded concept would influence the research on BE in the following years. In the 70s', the association between BE and gastroesophageal reflux disease and hiatal hernia was established, but the lack of adequate endoscopic tools, the presence of esophagitis and the concept introduced previously by Hayward, hampered the correct diagnosis and characterization of BE. The type of mucosa in BE was a matter of debate until 1976, when Paull and colleagues described the presence of 3 different types of columnar epithelium in the distal esophagus: cardia type, fundus type and intestinal type epithelium (7). In 1983, Skinner introduced the concept of 3 cm long Barrett's mucosa as an inclusion criterion in research studies (8). This cut-off started also to be used for diagnostic purposes in the daily clinical practice, leading to a widespread underdiagnoses of BE. The intestinal type mucosa was subsequently found to be the most predominant in BE, and to be strongly associated with the presence of dysplasia and carcinoma. The fact that intestinal metaplasia (IM) was clearly distinguishable from normal

gastric mucosa and considered a marker for the progression to adenocarcinoma, lead to the definition of BE by the presence of IM. In the 90s', endoscopic studies detected the presence of IM in columnar epithelium less than 3cm long, even in patients without symptoms (9). This lead to a shift in Barrett's diagnosis, and the concepts of long segment (>3cm) and short segment (<3cm) BE were introduced. However, a controversy remains on the role of IM detection for the diagnosis of BE.

1.2 **PATHOGENESIS**

Conceivably, changes in cell programming lead to modification of cell phenotype towards Barrett's metaplasia. Different origins for the progenitor metaplastic cells have been proposed, namely distal esophagus, cardia and bone marrow (10, 11). This transformation process may result from transdifferentiation, in which a fully differentiated cell, like squamous cell, changes phenotype into another kind of cell that was present in the esophagus during embryogenesis, such as columnar cell. Another proposed process is transcommitment, in which a stem cell in the esophagus would differentiate into columnar cell type, instead of differentiate into squamous cell type. The stem cells origin in transcommitment has been a theme of debate. Some authors propose origin in the esophagus (basal layer of squamous epithelium or submucoal glands), others extension from gastroesophageal junction, migration from gastric cardia or migration from circulating bone marrow stem cells (12). Regardless of the process of phenotype transformation, it is presumed that exposition to acid and bile acids trigger inflammatory processes, which activate signaling pathways and changes in key development transcription factors. These phenotypic changes start on a cellular level, leading later to tissue transformation, or metaplasia. There is some evidence supporting that columnar gastric type mucosa develops first. Further reprogramming leads to intestinal differentiation and later to goblet cells formation.

1.3 **EPIDEMIOLOGY AND RISK FACTORS**

The use of different diagnostic criteria and lack of symptoms in many BE patients may partially explain the disparity in results among studies on BE epidemiology. Early autopsy reports had suggested the presence of long segment BE in 0.4% of the population (13). More recently, population-based studies in adults established the prevalence of endoscopically suspected BE to be 2% in the East and 4-10% in Europe. When histology with IM was added as criteria for diagnosis, BE prevalence decreased to 1.3-1.6% in Europe (1/3 long segment and 2/3 short segment) (14).

Endoscopy based studies report a dramatic increase in the incidence of BE in the last decades. This may be partially associated with the increase in the number endoscopic procedures in that period. However, even after control for this increase in endoscopic practice, studies confirmed a rise in the incidence rates in the range of 100-159% (15, 16), with higher increases in younger ages (<50 years-old). That rise may also be related to better awareness among endoscopists and pathologists of this entity. Demographic data for patients with short segment and long segment BE are similar, indicating that these may be a continuum of the same process (14).

The risk factors for the presence of BE are chronic gastroesophageal reflux disease (GERD), age over 50 years, male gender, central obesity, smoking, Caucasian race and family history of BE (17). Most of these are also risk factors for esophageal adenocarcinoma (EAC).

Globally, 5-15% of patients with chronic GERD (> 5 years) have BE (17). That risk is more related with the duration (18) than with the severity of symptoms (19, 20). However, when considering BE subpopulations, the risk is fivefold for long segment but not significant in short segment BE (21). That limits the role of some screening strategies based on symptoms that can only identify approximately 45% of long segment BE, which constitutes a large subgroup of BE patients. Compared to other GERD patients, BE patients tend to have more frequently hiatal hernia, decreased tonus of lower esophageal sphincter, weaker distal peristalsis, and longer and more intense acid reflux (22, 23). However, it is interesting to notice that 40% of patients with EAC had no previous history of GERD (24).

BE prevalence increases with age, reaching a plateau at the 6^{th} -7^{th} decades (14). The length of BE is stable over time and is neither related to age nor to the presence of esophagitis (14). There is a male:female ratio of 2:1-3:1 in Barrett's prevalence in most studies, being that ratio higher in Caucasians (25, 26). This male predominance is higher at an earlier age, attaining values close to 4:1. In fact, BE may develop in males 20 years earlier, which may also explain the increase rate of EAC observed in males (27). Accordingly, the incidence of EAC in women is low, corresponding only to 12% of all EAC. Even among women with weekly GERD symptoms, the incidence of EAC is similar to the incidence of breast cancer in men (28). Prevalence of BE is 4-5 times higher in white populations compared to non-white individuals, irrespective if studies are from the same or from different countries (26, 29). That may be explained not only by other risk factors, such as visceral obesity and GERD, but also by genetic and epigenetic factors (30).

Obesity is a well-known risk factor for GERD and EAC. Increase in BMI is associated with increased risk of GERD, BE and EAC development (31, 32). It has been suggested that obesity plays a central role in GERD, through increase in intra-abdominal pressure, in the frequency of transient lower esophageal sphincter relaxations, in hiatal hernia prevalence, and through rise in inflammatory markers (32). In the last decades, obesity, BE and EAC have been increasing dramatically in the West. However, the initial increase in EAC preceded the emergence of the obesity epidemics. Also, obesity is increasing rapidly in groups with low risk of BE and EAC such as women and black people. Therefore, the increase in obesity *per se* cannot fully explain the rise in BE and EAC. Visceral obesity may help to explain the gender and ethnic differences observed in BE epidemiology (33, 34). A recent meta-analysis suggested that patients with visceral obesity have an increased risk for BE compared to non-obese individuals (OR, 1.98; 95% CI, 1.52-2.57). That relation was present even after adjustments for BMI and GERD (35). It may be assumed that the increase in obesity, namely visceral obesity in some groups, may lead to a future increase in the incidence of GERD, BE and EAC.

Helicobacter pylori is a known risk factor for atrophic gastritis, a condition that causes reduction in the gastric acid output and in gastroesophageal reflux (36). Decreasing *H. pylori* infection, particularly Cag A+ strains, may be one of the contributing factors for the described increasing incidence of BE and EAC (37, 38). This inverse association is emphasized by the fact that BE and EAC are still rare in countries with high incidence of *H. pylori* infection. This hypothesis may also be supported by the observed presence of cohort effects in *H. pylori* infections and BO incidence (39, 40). The effect of *H. Pylori* eradication in infected BE patients is not known. Moreover, the hypothetical role of H. pylori, does not explain the gender and ethnic differences observed in BE and EAC.
Alcohol intake has been a theme of debate but seems to not confer additional risk for BE. Conversely, studies had suggested that the intake of alcohol, namely wine, may be protective with ORs ranging from 0.44 (95% CI 0.2–0.99) to 0.71 (95% CI 0.52–0.98) (41). Smoking

has been associated with increased risk for BE (OR 1.4, 95% CI 1.2-1.7). That relation is present comparatively to non-GERD controls, but absent in the lack of GERD, suggesting an effect of tobacco mediated by increase in GERD (42).

1.4 DIAGNOSTIC CRITERIA

Precision and accuracy of diagnostic criteria are essential for proper study and management of a disease. Large cohort studies have established the low risk of cancer progression in BE without IM (43). That assumption accounts for the fact that in the United States and most European countries, the presence of IM on biopsy specimens has been a requirement for BE diagnosis. However, other studies suggested that the risk for cancer development is also present when IM is absent (44). Lack of IM has been associated with sampling error, being diagnosis of IM more frequent with increasing number of biopsy samples (45). These findings had led to changes in the diagnosis criteria of BE. First, the British Society of Gastroenterology proposed the diagnosis of BE by the presence of columnar lined esophagus on histology (46). Later, the American Gastroenterology Association Institute's recommended the definition of BE, as "the condition in which any extent of metaplastic columnar epithelium that predisposes to cancer development replaces the stratified squamous epithelium that normally lines the distal esophagus." (47). However, recent guidelines from the American College of Gastroenterology still require the presence of IM for BE diagnosis. That is done assuming the low risk of cancer progression and the negative impact in quality of life and insurance status of BE diagnosis (17).

1.4.1 Histology

Esophageal histology is characterized by the presence of stratified squamous epithelium and submucosal glands. Columnar epithelium above the anatomic gastroesophageal junction is metaplastic and considered a consequence of chronic inflammation. The superficial metaplastic epithelium may show features of gastric, intestinal or squamous cells. The deep glandular components may also exhibit mucous and/or oxyntic phenotypes. The reason for such heterogeneity is not known, but probably is related to the stage of BE progression. This metaplastic change is also associated with mesenchymal transformation with duplication of muscularis mucosae layer and development of blood and lymphatic vessels (48).
The presence of goblet cells is the landmark of intestinal metaplasia. Those are well differentiated nonproliferative cells that secrete mucins. Their presence is a *sine qua non* condition for the diagnosis of BE according to some international guidelines. Inflammation due to gastroesophageal reflux or H. pylori infection may act as a trigger for IM transformation in the esophagus and stomach, respectively.

There are 3 main factors in histological evaluation of BE: 1) identification of goblet cells in the columnar epithelium for the diagnosis of IM; 2) differentiation between esophageal IM and gastric IM in the distal esophagus; 3) grading of neoplastic changes in the Barrett's mucosa.
Diagnosis of IM demands the presence of goblet cells, but sometimes it may be difficult to differentiate them from pseudoglobet cells (49). In this regards, special stains seem to add no additional information to that obtained by conventional hematoxylin and eosin (H&E). Pseudogoblet cells are columnar, disposed in rows, have barrel-type shape and lack the typical triangular nucleus of goblet cells. The former have distended cytoplasmatic vacuoles that, like true goblet cells, have acidic mucin leading to the typical blue color on H&E, and a lighter blue color on Alcian blue stain than goblet cells (Figure 1 a-b). Even among expert

GI pathologists, the interobserver agreement for diagnosis of true goblet cells is low (50). The number of goblet cells in Barrett's mucosa increases with the number of biopsies taken, Barrett's length, male gender, white race and increased age, being rare in pediatric population (45, 51). Some studies reported increased number of goblet cells in proximal esophagus, while others describe a random distribution along the columnar epithelium. It was recently proposed, that the level of intraluminal pH and the effect of pH on bile acid dissociation may affect the density of goblet cells (52).

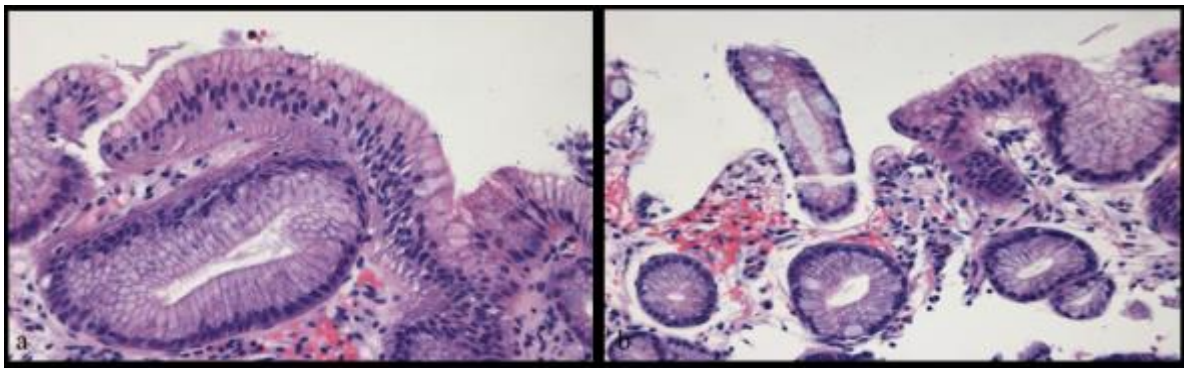


Figure 1. Histopathological (H&E) examples of columnar lined esophagus with pseudogoblet cells (a) and goblet cells (b). Courtesy by Michael Vieth.

It is difficult to distinguish endoscopically between an irregular Z-line and an ultrashort BE. Histologically, that differentiation is also difficult but some morphological features may be indicative of esophageal origin, such as presence of buried glands, esophageal glands or ducts, and multilayered epithelium. However, biopsy sampling of normal appearing or slightly irregular distal esophagus to look for IM is not recommended (17) . When there are endoscopic doubts and clinical value regarding the presence IM in the distal esophagus, gastric biopsies may be useful, since those may confirm or rule out diffuse gastritis with IM.

Esophageal adenocarcinoma is a result of a multistep process starting in columnar metaplasia, passing through different stages of dysplasia until the development of carcinoma (Figure 2.a-d) (49). Dysplasia is defined by the presence of neoplastic epithelium up to the basement membrane. Different dysplastic phenotypes such as intestinal, gastric or serrated may be present in Barrett's mucosa. Intestinal type is the most frequently associated with dysplasia, but gastric (foveolar) type may be present in up to 8% of all BE-associated dysplasia cases (53, 54). Irrespectively of type, dysplasia may be graded as negative, low or high-grade, according to cellular and architectural changes. The presence and grade of dysplasia is the major risk assessment tool in BE. But its important clinical value is hampered by inherent limitations of histological assessment. Morphologically, differences between low-grade (LGD) and high-grade dysplasia (HGD) and between HGD and carcinoma may be difficult to access and are not completely scientifically validated. In the West, the concept of intramucosal carcinoma defined by invasion of lamina propria that does not pass through the muscularis mucosa is widely used, but there are still no validated criteria for invasion of lamina propria. There is appreciable inter-observer variability among expert and non-expert GI pathologists in grading dysplasia in BE. That agreement may be reasonable in cases of no dysplasia or HGD/cancer (55) but is suboptimal (poor to fair) in cases indefinite for dysplasia or LGD even among expert pathologists (56). Community pathologists tend to overdiagnose LGD (57). In fact, expert and consensus evaluation leads to down-staging in 85% of LGD diagnosed in community settings. This poor agreement may be amplified by the presence of inflammation that is frequent in BE and that can cause regenerative changes that mimic dysplasia. In that case, the designation "indefinite for dysplasia" may be used as a provisionary diagnosis. It should be taken in account that "indefinite for dysplasia" is also a

diagnosis hampered by low inter-observer agreement. In fact, it is associated with kappa (κ) values of 0.18, even lower than the observed for LGD (0.35) (58). Studies in patients with the diagnosis "indefinite for dysplasia", have reported risk for cancer progression similar to LGD (59), being that risk higher when findings are multifocal (60). Other studies describe a high risk for progression during the first year after the diagnosis of "indefinite for dysplasia", but thereafter a risk similar to non-dysplastic BE (61).

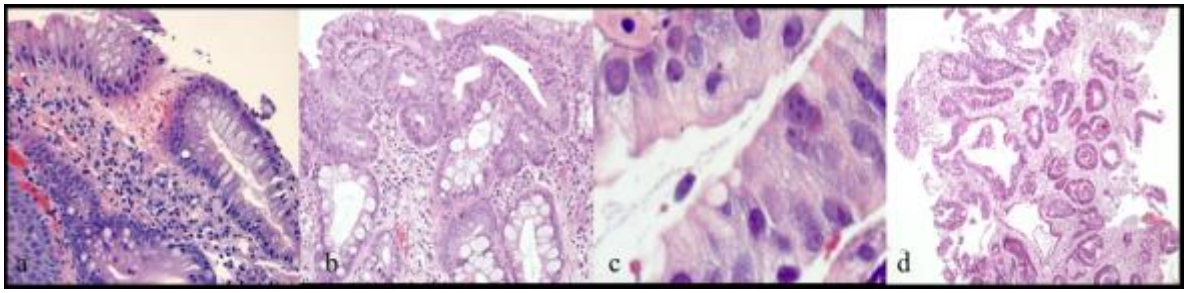


Figure 2. Histopathological (H&E) view of BE multistep progression from non-dysplastic intestinal metaplasia (a), to low-grade dysplasia (b), high-grade dysplasia (c) and later adenocarcinoma (c). Courtesy by Michael Vieth.

Finally, different subtypes of metaplastic epithelium, as mentioned above, may be unrecognized leading to understaging in some cases. Due to limitations of conventional morphology in the grading of BE, complementary evaluation with different markers have been explored. Markers of differentiation such as CD10, proliferation markers like Ki67, genetic mutations such p16, p53 and Kras, study of DNA content (aneuploidy/tetraploidy) and enzymes like COX-2, have been extensively evaluated. However, their value is variable among studies and limited by low accuracy. Until now, conventional histomorphology continues to be the gold standard for BE characterization and grading (47).

1.4.2 Endoscopy

Irrespective of histological criteria, endoscopy is necessary for BE diagnosis. The aim of endoscopy is to detect, not only Barrett's mucosa, but also to identify early lesions suitable for curative treatment. Endoscopically, Barrett's mucosa is characterized by the presence of columnar-salmon-like mucosa in the distal esophagus (Figure 3.a). An accurate characterization of the gastroesophageal junction (GEJ) as the transition between the tubular esophagus and the upper limit of the gastric folds is essential for proper diagnosis and classification (62). All columnar epithelium extending proximally to this junction may be defined as BE. Extensions longer than 3cm are called long segment, between 1-3 cm as short segment, and <1 cm as ultra-short segment BE (Figure 3.b-c). Some describe ultra-short BE as "IM of the esophagogastric junction", due to high inter-observer variability and low cancer risk (17, 63).

Before inspection, mucosa surface shall be clean of mucus and debris using water or a mucolytic agent. Then, the main endoscopic landmarks, the hiatal hernia, gastroesophageal and the squamocolumnar junctions should be assessed, and a careful retroflexed view of the GEJ should be performed. Respiratory movements, esophageal and gastric motility, and endoscopic air insufflation may influence proper assessment of GEJ. For that reason, some authors proposed the end of esophageal palisade vessels as a landmark for GEJ. But later studies had ruled out this landmark due to lack of accuracy (64). In case of esophagitis, 8 weeks' treatment with proton pump inhibitors (PPIs) should be performed, as it may be

otherwise difficult to define the Barrett's extent and evaluate its morphological changes, including the degree of dysplasia.

The circular and maximum extension of Barrett's mucosa shall be classified according to the Prague criteria that have shown to be accurate in different contexts (62, 65) (Figure 4.a). The circular (C) and maximum (M) extent of the columnar mucosa shall be measured starting at the oral end of the gastric folds and moving proximally the endoscope. Measurements are made using the scale on the shaft of the endoscope and excluding any squamous islands. This standardization is pivotal for comparisons in the follow-up endoscopies, but it also facilitates communication between endoscopists, being also important for research purposes. These criteria proved to have high κ values of 0.90 (95% CI, 0.80 - 1.00) and 0.92 (95% CI, 0.87 - 0.98) for the C and M values, respectively, when BE ≥ 1cm (62). Despite these benefits, the Prague criteria are used by only 22% of gastroenterologists (66).

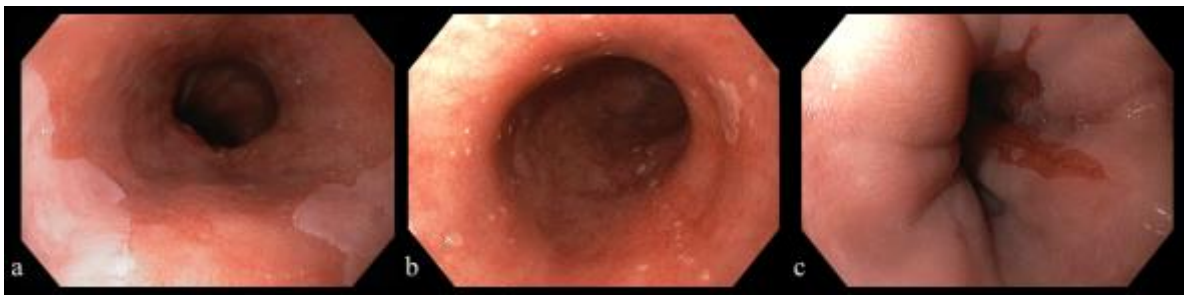


Figure 3. Endoscopic view of the distal esophagus with salmon-colored Barrett's mucosa above the end of gastric folds. A proximal displacement of the squamous-columnar junction can also be seen (a). Long-segment BE with squamous islands (b). Short segment BE (c).

During the endoscopic investigation, Barrett's mucosa shall be carefully evaluated in order to detect lesions. When detected, macroscopic lesions should be fully characterized using the Paris classification (Figure 4.b) (67). Good characterization is essential before proper management is decided, because macroscopy is related with invasion depth, which is associated with risk of metastasis. The use of these criteria/classification systems and enhanced endoscopy demands longer evaluation time. This increase in evaluation time is associated with increase in the neoplasia detection rate, being actually recommended an evaluation time of at least 1 minute per cm of Barrett's mucosa (68). Special attention shall be given to the right hemisphere of the mucosal lining (from 12 to 6 o'clock position), where the risk of neoplasia is higher (69). Detection of lesions may be difficult due to the presence of different types of mucosa within the BE and the presence of multifocal neoplasia. Even when lesions are observed, their full characterization and delineation may be troublesome. Automated endoscopic detection systems may evolve as a valid tool for clinical and learning purposes (70).

Conventional endoscopy has some limitations in lesion detection, where high-definition imaging has been found to be superior (71, 72). New endoscopic techniques have been developed aiming to facilitate the recognition of neoplasia by enhancing mucosal morphology (i.e., mucosal and vascular patterns). These techniques intend to act as a "red flag tool" to improve neoplasia detection, which shall be followed by further characterization.

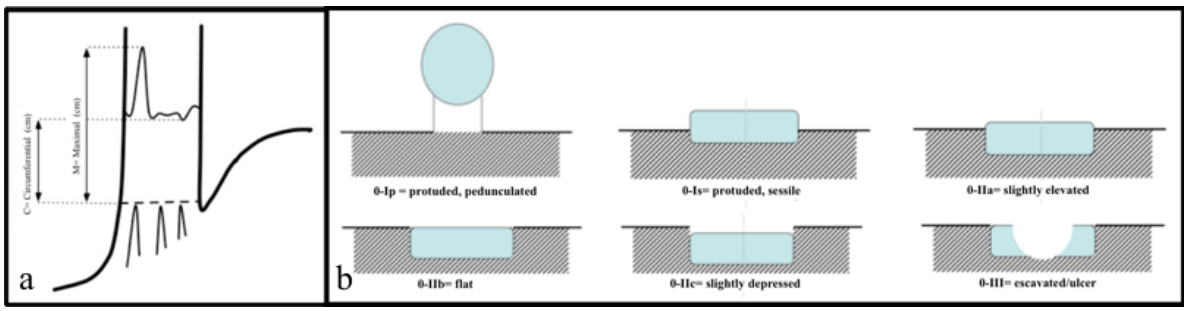


Figure 4. Classification of BE according to the Prague (C&M) criteria (a). Paris classification of gastrointestinal neoplasia (b).

A wide variety of image enhancement techniques have been studied including conventional chromoendoscopy, virtual chromoendoscopy, autofluorescence endoscopy, confocal laser endomicroscopy, volumetric laser endomicroscopy, spectroscopy and molecular imaging. Conventional chromoendoscopy uses dyeing agents to provide mucosal contrast enhancement and thereby better characterization. Vital stains such as methylene blue are retained by absorbing cells like the ones present in the colonic or small bowel epithelium. Within BE, methylene blue highlights areas of intestinal metaplasia and will not stain areas of gastric metaplasia or islands of squamous epithelium. Contrary, contrast stains like indigo carmine are not absorbed by cells and accumulates in the surface of epithelium, highlighting mucosal patterns. Acetic acid is another commonly used chromoendoscopy agent. It disrupts the superficial mucus layer and induces protein denaturation. This causes an aceto-whitening reaction that masks the submucosal capillaries and increases the opacity of the mucosal surface, highlighting the surface pattern. The presence of focal erythema after loss of aceto-whitening may be a sign of neoplastic transformation (73).

Virtual chromoendoscopy uses light filters or post-processing imaging in order to improve the characterization of mucosal morphology. Narrow Band Imaging (NBI) is a widely disseminated endoscopic technology that applies spectral narrow band filters in the endoscope lighting (Figure 3.a). This technology is based on the fact that the depth of light penetration into the tissues is related to its wavelength. In NBI, an increased amount of blue light is used for lighting of the endoscope. As blue light has a relatively short wave length, it penetrates more superficially into the tissues. That enhances the imaging of the superficial epithelial layer (figure 3.b-c). Moreover, blue light is highly absorbed by erythrocytes, which enables a better visualization and characterization of the superficial vasculature (74).

Different groups have proposed several classification systems for BE characterization using NBI (75-77), However most of these classifications are complex and the real value of NBI per se has been debated (78). A randomized controlled trial (RCT) comparing NBI with high-definition white light endoscopy (HDWLE) revealed no differences in the detection rate per patient irrespective of the method used, albeit that NBI demanded fewer biopsies (79). Despite the fact that in this study, regular appearing NBI surface patterns did not have HGD or cancer, the authors concluded that routine use of NBI targeted biopsies for detection of HGD/cancer was not recommendable. It must also be mentioned that even HGD may be present in deep mucosal layers with normal appearing superficial mucosa (80). A recent study using a simplified NBI classification reported 85% overall accuracy, 80% sensitivity, 88% specificity, being accuracy of 92% if the experts were confident in their prediction, with substantial inter-observer agreement ($\kappa = 0.68$) (81). But it must be taken into account that this was not a per-patient evaluation, contained no cases of LGD, but instead 37% of lesions had HGD/cancer. In addition, only still pictures were analyzed and all raters were experts in the field. Accordingly, this study hardly reflected the prevailing situation in routine

endoscopic clinical practice. In fact, only 31% of endoscopists in the US use enhanced endoscopy for selective investigations (66).

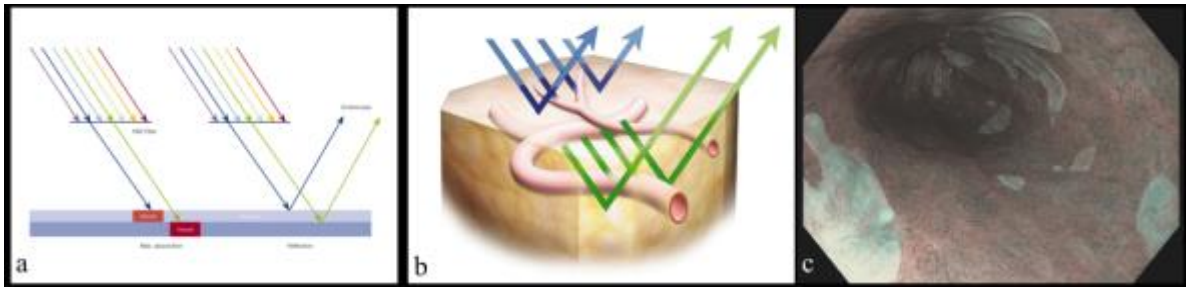


Figure 5. Narrow Band Imaging uses filters that select the blue and green lights (a), that due to its wavelengths penetrate the superficial mucosal layer and vasculature (b). Endoscopic view of a long-segment BE, with mucosal and vascular enhancement in Barrett's epithelium and pale mucosa in squamous epithelium (c).

After full characterization, biopsies shall be performed in suspicious areas of Barrett's mucosa. Even a subtle change in the mucosa, such as an erosion, nodule or small irregularity shall be biopsied due to risk of underlying neoplasia (82). It is important to notice that even biopsy sampling could lack accuracy in characterizing these lesions. In fact, it has recently been confirmed that resection of macroscopic lesions may lead to histological downgrade and upgrade of 16% and 23% of the lesions, respectively (83). Resection is also associated with better inter-observer agreement among pathologists as compared to material harvested through biopsy forceps (84).

After careful endoscopic evaluation with biopsies from macroscopically lesions, random 4-quadrant biopsies shall be performed every 1-2 cm according to the Seattle protocol (85, 86). This systematic approach is associated with increased detection rate of neoplasia, comparing to random *ad hoc* biopsies (87). The Seattle biopsy protocol is time-consuming, costly, carries the risk of sampling error, and is hampered by low compliance (88), especially in long segment BE (89). This strategy may sample less than 5% of Barrett's mucosa (72). However, it has shown to be superior to targeted biopsies (87, 90). If at the index endoscopy, at least 8 biopsy specimens are taken from Barrett's mucosa, there is no need for confirmatory investigation concerning diagnosis of BE (17, 45). However, a new endoscopy with biopsies may be considered after 1-2 years in cases of no IM found, despite an appropriate number or biopsies taken (17). Still, with that approach, 70% of these patients will remain negative for IM (91).

A recent meta-analysis suggested that enhanced endoscopy could increase detection of dysplasia, without no significant yield differences between virtual and conventional chromoendoscopy (92). One of the major problems with current technologies relates to the spatial resolution. The level of magnification and the details needed for complete mucosal assessment are not adopted to the task of assessing such a wide surface area present in the majority of patients with BE.

1.5 CLINICAL COURSE

The clinical manifestations and eventual progression of BE vary substantially between individual patients. Another complicating factor to comprehensively understand the natural course of BE resides in the fact that BE patients are often asymptomatic and do not seek

medical care. BE results from chronic GERD, and most of symptoms associated with it are a consequence of reflux. GERD-related symptoms are present in 80% of long segment BE and in 45% of short segment BE patients. BE is the major predisposing factor for EAC development. EAC continues to be a cancer with low prevalence, but its incidence has increased dramatically in the West in the last decades (93).

BE is the only known condition that predisposes to EAC, but BE patients in general have low mortality attributed to EAC, with a global incidence of 0.2-2.9%. In fact, less than 5%, of patients with EAC have a prior diagnosis of BE (94, 95). Most of BE patients have significant mortality due other causes than EAC, such as cardiac disease (35%) and pulmonary diseases (20%), being only 7% related to EAC (96). That may be explained by the advanced age of BE patients and comorbidities associated with aging.

The diagnosis of EAC is associated with a poor prognosis, carrying a 5-year survival of less than 20% (97, 98). Risk factors for the progression of BE into EAC are the presence of esophagitis, Barrett's length, more than 10 years after BE diagnosis, and presence of dysplasia at index endoscopy (99). The risk for cancer progression in long, short and ultra-short BE is 0.22%, 0.03% and 0.01%, respectively. In fact, in patients with T1 EACs, 56% have LSBE, 24% SSBE and 20% ultra-short-segment BE (100). A risk for progression into cancer of 14% per cm increase in Barrett's length has been reported (99).

The presence and grade of dysplasia in Barrett's mucosa is associated with an enhanced risk of progression into EAC. In the absence of dysplasia, the incidence rate of EAC is 0.33%, being lower (0.19%) when only short segment BE is considered (101). In the presence of LGD and HGD that risk is 0.54%-1.73% and 7-19%, respectively (101-104). Estimates of progression rates based on dysplasia scoring have been modified in the recent years. The previous overestimation could be related to the absence of short segment BE in studies earlier than 1994, lack of medical therapy in most patients, and pathological overstaging. It is now known that the certainty in the histological diagnosis of dysplasia, reflected by pathologist expertise and inter-observer agreement, is associated with increased risk of progression. Many previous studies lack pathologist agreement or expertise for diagnosis of dysplasia. That may well have had an impact on the updated calculations of the risk of progression. Also, the exclusion of prevalent cases of EAC (diagnosis within 3 years after index endoscopy) leads to a marked decrease in the risk estimates of BE progression.

1.6 **SCREENING**

Screening is defined by a systematic application of a test to identify individuals at risk for a specific disorder, to warrant further investigation or direct preventive action (105). A screening program is considered suitable, if the condition can be detected in early stages and has significant impact in society, if screening shows to reduce the burden of the disorder and if it is cost effective. Screening in BE aims to detect not only BE patients for subsequent surveillance, but also to identify patients with dysplasia or early cancer, suitable for curative treatment. In fact, endoscopic therapy has proven to reduce mortality in patients with early Barrett's cancer (106).

In order to reduce costs of a general screening program and subsequent surveillance, economic modeling studies have proposed a target population for endoscopic screening: more than 50 years-old males with chronic (>5 years) GERD (107, 108). Several modalities may be applied for screening purposes. The endoscopy based modalities include

conventional endoscopy, ultrathin endoscopy and capsule endoscopy. Non-endoscopic modalities include cytosponge and blood tests on genetic susceptibility, micro RNA, proteome and metabolome analyses (109).

Conventional upper endoscopy is the most studied screening modality, but it is relatively invasive and expensive and may be associated with significant "sampling error" (risk to miss changes in endoscopic biopsy sampling) as well as "diagnostic errors" (difficulty to obtain a correct histopathological diagnosis including grading of dysplasia), which all limits its value (110). Conventional endoscopy may be associated with BE overdiagnosis in 32% of cases in clinical practice (111). However, in case of a negative endoscopy for BE, a subsequent endoscopy has limited value, leading to an increase in diagnostic yield of 2%, being that increment higher in cases of esophagitis (112). Some studies have proposed a role for ultrathin endoscopy in BE screening (113, 114). Its use is well tolerated, safe and associated with reduced costs due to lack of sedation, but may have a lower yield when it comes to biopsy sampling (80%). Although ultrathin endoscopy is not yet widely available, it may be potentially performed by non-physician providers, increasing its yield.

Capsule endoscopy has been proposed as a screening tool, due to lack of sedation and patients' good acceptance. However, conventional endoscopy seems to be more cost effective (115, 116). Capsule endoscopy is associated with lower accuracy (78% of sensitivity and 73% of specificity) and does not enable biopsy sampling (117). Cytosponge is a new cytology acquisition device that is swallowed by the patient into the stomach and then pulled out using a string, collecting cells along the esophagus. It does not require sedation nor need to be performed by a physician. Cytology analysis testing for trifoil factor 3 was shown to have sensitivity and specificity rates for BE diagnosis of 73% and 94%, respectively (118). This method may be associated with low participation rate in a screening context (18%), but is be well tolerated, and cost effective in a modeling study (118, 119). The study of circulating microRNA and of proteome and metabolome panels may be future noninvasive tools in BE screening, but their use in in BE and EAC has been limited so far (120).

Most of cost-effectiveness modeling studies on BE screening are based on old data harboring an overestimation of the BE progression, so the role of screening may be overvalued. Although BE is more frequent in GERD patients, 44% of BE patients don't have GERD related symptoms (121). Only 5% of EACs patients have a previous diagnosis of BE (94), meaning that the value of the detection of EAC precursors at an early stage is reduced. Predictive scores that combine different risk factors may lead to a more targeted screening program, increasing its value (122). Currently, most of international guidelines do not recommend BE screening in general population, but they suggest that screening may be considered when multiple risk factors for EAC are present (17, 47, 123).

1.7 **SURVEILLANCE**

In the Barrett's context, the aim of surveillance is to detect neoplastic changes in an early stage suitable for curative treatment. Most of the factors discussed in screening can also be applied in the discussion on BE surveillance. In patients with EAC, histological grade and depth of neoplastic invasion determine the lymph node metastasis risk and survival (124). Data from observational and retrospective studies suggest increased disease-specific survival in patients undergoing BE surveillance programs. But data is scarce and may be impaired by "publication bias" and "referral bias". More recent data show conflicting results. Two large European population studies found that patients under adequate endoscopic surveillance or

with previous diagnosis of BE have EAC detected at early stage with improved survival (125, 126). An American case-control study did not support the benefits of endoscopic surveillance in terms of survival of EAC (127). However, the results from this study may be criticized based on the fact that among patients with EAC that went through surveillance, 40% did not have an endoscopy in the previous 5 years, and only 11% had intramucosal cancer. Furthermore, in this study there is no mention to systematic endoscopic procedures or biopsy sampling protocol.

The lack of prospective trials, together with recent data suggesting that the risk for EAC development in non-dysplastic BE patients is lower than previously reported, resulted in increased controversy about BE surveillance, particularly in short segment BE (101, 128). Evidence is weak, but most current recommendations favor surveillance (17, 72, 129). It has to be recognized that surveillance is demanding for patients with reflection in their perceptions on prognosis and quality of life (130-132). Enough space should be left for individualization, depending on patient's preference, age and clinical condition. Patients shall be well informed about the risk for the development of EAC, the limitations of surveillance and possible treatments in case neoplasia is found.

High-definition/high-resolution endoscopes shall be used in BE surveillance (17). Some recent studies and meta-analyses favors the use of chromoendoscopy or virtual chromoendoscopy in BE (92, 133). Despite the marked increase in endoscopic imaging quality, most international guidelines still recommend surveillance with systematic 4-quadrant biopsies each 1-2 cm (17, 72, 129). However, this strategy is costly and exposed to sampling error and low adherence among endoscopists. Presence and grade of dysplasia remain the best risk predictors for cancer progression in BE, which influences the surveillance strategy. In fact, the presence of any grade of dysplasia should be confirmed by 2 expert pathologists (129, 134).

The paucity of evidence leads to a considerable variability in proposed surveillance strategies among the different gastroenterological societies. For patients with columnar lined esophagus shorter than 3cm, without IM or dysplasia, a repeat endoscopy with quadrantic biopsies is recommended to confirm the diagnosis (72). If, after a detailed and repeated biopsy protocol, no IM or dysplasia are found in a short segment BE, surveillance may be stopped (129); in the presence of IM without dysplasia, most current guidelines propose surveillance endoscopy after 3 to 5 years (17, 129, 135); in the case of BE indefinite for dysplasia, effective acid suppression followed by new endoscopy in 3-6 months is advocated (17). The presence of dysplasia shall be confirmed by an expert BE pathologist and should be followed by endoscopy with removal of macroscopic lesions and four-quadrant biopsies every 1 cm (17). Also, in the case of pathologically confirmed LGD, a repeated endoscopy after optimized PPI therapy is recommended. If LGD is present at the second endoscopy, and no endoscopic therapy is performed, endoscopy surveillance is proposed every 6-12 months until 2 consecutive endoscopies do not reveal presence of dysplasia (17, 129). In case of morphologically confirmed HGD, endoscopic resection of all visible lesions and full mapping shall be performed to maximize the staging. A general recommendation is that these patients shall be referred to expert centers.

1.8 **PREVENTION**

Prevention aims to avoid or delay neoplastic transformation of Barrett's mucosa. Most studies in this field have been focused on reducing the exposure of Barrett's epithelium to

deleterious gastroduodenal content. Other approaches aim to directly reduce the inflammatory and proliferative triggers in Barrett's mucosa. The significance and role of these different strategies are difficult to ascertain due to the low rate of progression of non-dysplastic BE, and to the use of endoscopic ablative and resection therapies in neoplastic BE that decrease the pool of patients that would benefit from preventive approaches.

1.8.1 Chemoprevention

Acid suppressive drugs

Most BE patients have GERD-related symptoms and are given long-term PPI therapy (17). While in symptomatic BE patients the use of PPI is consensual, some controversy remains in the preventive use of PPI in asymptomatic patients. PPI are effective drugs that reduce gastric acid secretion and, through that, reduce acid-triggered inflammation in Barrett's mucosa. Acid-induced injuries are considered the major factor for BE formation and for its neoplastic transformation. PPI therapy is widely available, is considered safe and its costs have decreased dramatically in the recent years (136). The combination of these factors may justify the use of PPI even in asymptomatic BE patients. But most of the data available on this topic is based on expert opinions and not on RCTs. While most prospective and retrospective studies had shown a protective effect of PPI use in neoplastic Barrett's progression (137-139), two recent population-based studies failed to demonstrate such an effect (140, 141). A meta-analysis based on 7 studies suggested a risk reduction for the progression to HGD/EAC of 71% with PPI (OR 0.3; 95% CI 0.1–0.8), the effect being dose-dependent. In that study, no protective effect was observed in users of H2RA (histamine-2 receptor antagonists) (142). According to the conflicting nature of these data, some guidelines propose chemoprevention with a daily PPI dose (17), while others advocate its use only in symptomatic patients (129).

Anti-inflammatory drugs

The use of acetylsalicylic acid (ASA) or other nonsteroidal anti-inflammatory drugs (NSAIDs) has been associated with inhibition of several oncological pathways in different contexts. Their use has been extensively studied in the context of BE and EAC chemoprevention. Early studies report conflicting results, but a more recent meta-analysis describes a slightly inverse correlation between use of aspirin and NSAID with development of EAC (OR, 0.64; 95% CI, 0.52–0.79, and OR, 0.68; 95% CI, 0.56-0.83, respectively). Increased frequency and duration of drugs use were associated with a protective effect (143, 144). These drugs are widely available, but contrary to PPIs, their use may be associated with severe complications such as gastrointestinal (GI) and cerebral bleeding. Due to low risk of progression of non-dysplastic BE, the good results of endoscopic treatment of LGD in BE (145), and the risks associated with these drugs, their routine use is not recommended (17, 129). However, it is important to notice that cardiovascular disease is a prevalent condition in BE patients. In such patients, these drugs may confer additional protection, in addition to their cardiovascular role.

Statins

Statins are 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, used in primary and secondary prevention of cardiovascular diseases. In addition to improve blood cholesterol levels, these drugs may prevent cancer development and progression (146-149), but their role in BE remains controversial (150, 151). In fact, some studies favor their use in EAC prevention (147), while others show no beneficial effect (152). In a recent meta-

analysis, including 5 studies and 2125 patients, statins were associated with reduced risk for EAC of 41% (adjusted OR, 0.59; 95% CI, 0.45–0.78) with consistent results among all studies. The number of patients needed to be treated with statins to prevent 1 case of EAC in patients with BE was 389 (153). Current guidelines do not recommend its routine use in BE (17, 129).

1.8.2 Surgery

Anti-reflux surgery (ARS) has the potential to reduce both acidic and non-acidic reflux in BE patients, factors that are associated with progression and proliferation in Barrett's mucosa. A small RCT reported no differences in BE neoplastic progression in operated patients versus patients under medical therapy. This study included treatment with H2RA and PPI and was probably underpowered to detect differences in outcome (154). A later study showed a protective role of ARS in BE patients (155). This study had also some pitfalls, namely heterogeneous medical treatment, the inclusion of less than 50 operated patients, with a skewness towards younger ages in those allocated to surgery. Two meta-analyses and a systematic review highlighted the heterogeneity of the published literature and the lack of superiority of any of these strategies (101, 156, 157). ARS should be considered when GERD related symptoms or esophagitis cannot be controlled by medical therapy and has until now not proven to exert a preventive effect on neoplastic progression in BE (17, 129).

2 AIMS

The specific aims of the thesis were:

1. To determine whether acid reflux co-varies with symptom scores throughout the upwards titration of PPI dosing in BE patients, and whether this strategy could eliminate acid reflux in these patients.

2. To ascertain if PPI therapy can achieve the same level of acid reflux and symptom's control as clinically successful fundoplication.

3. To determine the morphological changes in the columnar and squamous epithelium, and whether these alterations co-vary with the acid reflux variables in the respective groups.

4. To evaluate and compare different NBI classification systems in the endoscopic assessment of BE.

5. To validate the Amsterdam NBI classification for BE and to study if a structured learning program can improve its accuracy and validity.

3 MATERIAL AND METHODS

3.1 PARTICIPANTS AND STUDY DESIGNS

In study 1, we ascertained the impact of PPI or ARS (fundoplication) in esophageal acid reflux variables, symptom scores, and morphological changes in the columnar and squamous esophageal epithelium. Successive patients with long segment BE that were enrolled for endoscopic surveillance, were invited to participate in the study. Fifty-eight adult patients without (group 1, n=27) or with ARS (group 2, n=31) participated in this prospective study. After the first visit, patients in group 1 started PPI (pantoprazole) in a daily morning dose of 40 mg for 8 weeks, followed by re-evaluation with ambulatory 24h pH recording, endoscopy and symptoms assessment. In patients with persisting pathologic pH values, the dose of pantoprazole was increased to 80 mg/day (40 mg twice daily) for another 8 weeks, and in those still not reaching the study endpoint of normalized acidic reflux, the dose was additionally increased to 120 mg/day (40 mg two or three times daily, according to pH results) for another 8 weeks (Figure 6). Beyond this maximum dose, adding oral H2 receptor antagonist (ranitidine 300 mg) for control of night-time heartburn was allowed. In cases of intolerance or incomplete response to pantoprazole, a switch to the same dose of esomeprazole was done. Patients in group 2 went only through the baseline investigations (Figure 6).

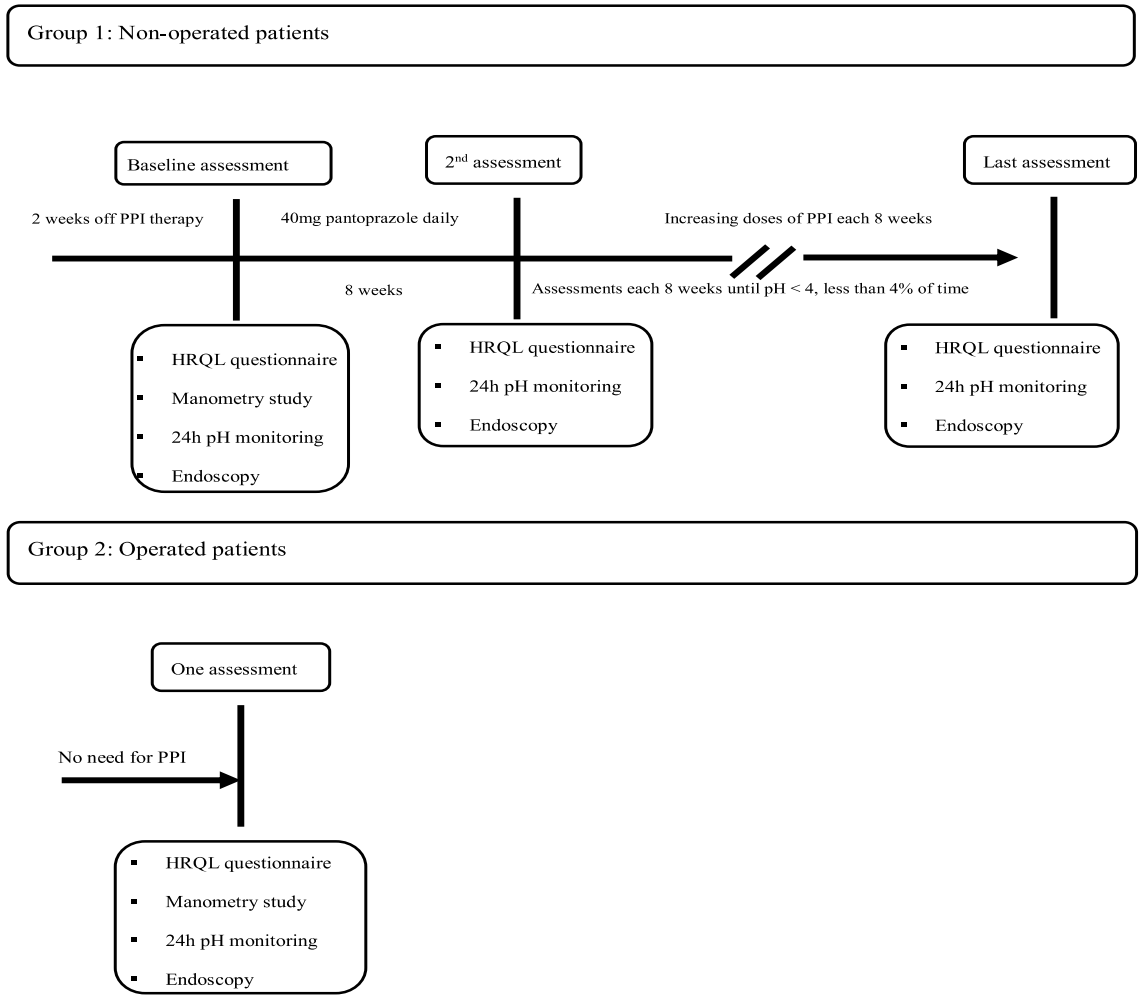


Figure 6. Flow-chart of study 1.

In study 2, we evaluated and compared different NBI classification systems in BE assessment. Patients with long segment BE were invited to participate. Thirty-two patients were included in the study.

In study 3, we assessed the role of the Amsterdam NBI classification system for BE, evaluating also if a structured learning program improved its accuracy. The 32 patients included in study 2 were also included in study 3.

3.2 ESOPHAGEAL MANOMETRY AND 24-H PH MONITORING (STUDY 1)

Stationary esophageal manometry was performed in each patient, at the first hospital visit, to define the location of the lower esophageal sphincter (LES). Thereafter, an ambulatory 24h pH monitoring was performed at each visit in group 1 (Figure 6). Patients were advised to maintain normal daily activities and to eat and sleep as usual. Symptoms, meals and postural changes were recorded by patients, using event markers on the data waist recorder. Intraluminal 24h pH monitoring was performed using dedicated pH electrodes (Versaflex, Alpine Biomed, Fountain Valley, CA, USA). In all assessments, one pH electrode was placed 5 cm above the LES. On each pH tracing, the percentage of total time with an esophageal pH<4, percentage in the supine and erect position, the total numbers of reflux episodes and the longest episode and the reflux index were analyzed. Complete acid suppression was considered to prevail when 24h esophageal pH in the distal electrode was inferior to 4 less than 4% of time.

3.3 HEALTH RELATED QUALITY OF LIFE (STUDY 1)

At each assessment, patients completed a gastroesophageal reflux disease-health related quality of life questionnaire (GERD-HRQL). This is a patient-centered questionnaire constructed to evaluate patient's perception of symptoms severity. It uses 10 questions graded in a 0-5 scale with a maximum score of 50, evaluating 4 main domains: intensity and frequency of heartburn, difficulty of swallowing, bloating and burden of GERD medication. This GERD-HRQL has been tested and validated in a wide range of patient groups, where higher scores reflect severe symptoms and worse quality of life (158).

3.4 ENDOSCOPY

All endoscopic investigations were performed by the same endoscopist (FBS) and digitally recorded. Examinations were performed with an endoscopic Olympus system (Olympus Corp., Tokyo, Japan), consisting of the ME-NBI endoscope GIF-Q160Z with magnification (maximal magnification, 115 times), a CV-180 processor and a CLV-180 light source. The tip of the endoscope was attached to the surface of the mucosa at each 2 cm at the 3 o'clock position, starting at the GEJ and ending at the distal squamous epithelium. In studies 2 and 3, a transparent cap was attached to the tip of the endoscope, enabling fixation of the endoscope to the mucosa while adapting the magnification mode and recording the videos. At each endoscopy, a systematic protocol was used and biopsy specimens were taken in suspicious areas, in the GEJ, and at the 3 o'clock position each 2 cm of BE and distal esophageal squamous epithelium. For tissue acquisition, standard biopsy forceps were used (Radial Jaw 3; Boston Scientific, Massachusetts, USA).

3.5 POSTENDOSCOPY ASSESSMENT (STUDIES 2 AND 3)

All video segments were anonymized and converted into AVI files using specific software (Pinnacle Studio, Mountain View, CA). Each video was randomly labeled and transferred to a computerized database. Videos corresponding to more than 1 histological type were excluded, leaving 3 main histological groups for assessment, i.e., gastric type mucosa, nondysplastic IM, and dysplastic IM. Quality of the videos was independently assessed by 2 experienced endoscopists (FBS, HUM). Only videos from flat mucosa and of good mucosal morphology quality, in which the subsequent video observation confirmed the targeting of the biopsies, were selected. In total, a group of 209 standardized, prospective, and different ME-NBI videos was collected. From these, 84 videos of 10 seconds in length were selected for subsequent evaluation, using simple randomization. The 84 videos corresponded histologically to gastric type mucosa (n=28), nondysplastic IM (n=29), and dysplastic IM (n=27).

In study 2, an education set was created with 15 videos not included in the evaluation set, corresponding to gastric type mucosa (n=5), nondysplastic IM (n=5), LGD (n=2), and HGD/EAC (n=3). Three different DVDs were created, 1 for each classification system. Every DVD consisted of one education and one evaluation set. To avoid bias from video recognition by the assessors, the same 84 videos were displayed in a random and completely new order for each DVD. The videos were labeled differently and sent to the observers at 3-week intervals in random order. Before starting the evaluation exercise, each assessor had to carefully study the educational set, which contained a description of the study and the corresponding classification system and the 15 educational ME-NBI videos.

In study 3, we selected the first 70 videos from the 84 randomly selected videos in study 2, corresponding 26 to gastric type mucosa, 23 to nondysplastic IM, and 21 to dysplastic IM.

3.6 EVALUATION OF VIDEO CLIPS (STUDIES 2 AND 3)

In study 2, nine endoscopists from 9 different University Hospitals in Europe and Japan participated in the study. Three were internationally well-known experts in the field of ME-NBI in BE (RK, KG, TR), 3 had expertise in BE but no particular experience with ME-NBI for BE (JH, AE, ET), and 3 had experience in ME-NBI in the stomach, but were unfamiliar with ME-NBI for BE (JS, MDR, MA). All observers were blinded to the histological and clinical data. Assessors studied each of the 15 educational videos and predicted the histology according to the principles of the corresponding classification system (75-77), i.e., as gastric type mucosa, nondysplastic IM, or dysplastic IM (Table 1). Observers also reported whether they were certain or uncertain about their predictions. The outcome and the duration of the procedures were recorded. Thereafter, the ME-NBI classification and histology were displayed for that particular video. The evaluation set included 84 videos displayed in a different and random order. Before classifying a video, the user could run it as many times as needed. Each video was then scored according to the respective classification system, including histological prediction and certainty of prediction. The time taken during these procedures was noted. If more than 1 endoscopic pattern was observed in the same video, the worst histological grade was considered. Contrary to the educational set, there was no characterization feedback.

In study 3, a software application was developed using Visual Basic 2010 (Microsoft Corporation, Redmond, USA), which was installed on each observer's computer. At the beginning, an educational set was displayed and carefully studied. This educational set

consisted of a PowerPoint presentation with a description of the Amsterdam classification, a video explanation of the software, followed by a series of 15 learning videos. Each participant could run each video as many times as necessary. After classification of each video, the assessors predicted the respective histology into one of the following categories: gastric type mucosa, IM, or dysplastic BE. At each site, assessors described whether they were certain or uncertain concerning the histological prediction. Then, the histological feedback was automatically given whereupon the access to that video was blocked. The same procedure was followed for each of the 15 learning videos and in each of the 70 evaluation videos. The time needed for each evaluation was automatically registered. Six endoscopists with different levels of ME-NBI expertise from four different University Hospitals in Europe and Japan participated. Three had extensive endoscopy practice but no previous experience from ME-NBI in BE (MM, PB, PP). The remaining three (KG, ET, JS) had extensive experience from these techniques and participated in the previous Barrett ME-NBI study (study 2).

In studies 2 and 3, all assessors were blinded to the endoscopic, histological, and clinical data.

Classification	Kansas	Amsterdam	Nottingham
Mucosal Morphology	Mucosal pattern: circular/ridge/villous/ irregular/ distorted	Mucosal pattern: regular/flat/ irregular	Type A: round/oval pits with regular microvasculature
			Type B: villous/ridge/linear pits with regular microvasculature
	Vascular pattern: normal/abnormal	Vascular pattern: regular/irregular	
			Type C: absent pits with regular microvasculature
		Abnormal blood vessels: absent/present	
			Type D: distorted pits with irregular microvasculature

Table 1. Mucosal morphology according to the 3 main classification systems described for BE characterization using magnification endoscopy with NBI.

3.7 HISTOPHATOLOGY

In study 1, all biopsy specimens were stained with H&E and analyzed by two expert gastrointestinal pathologists (MV & MD) that were blinded to patients' group affiliation, clinical history and to the endoscopy findings. The histological assessment of the squamous epithelium included scoring of basal cell layer and epithelial total thicknesses, papillary length, intercellular space dilation and number of inflammatory cells (neutrophils, eosinophils and mononuclear cells) accordingly to published guidelines (159).

In study 2 and 3, all biopsy specimens from Barrett's mucosa were analyzed by an expert gastrointestinal pathologist (MV) that was blinded to patients' clinical history and endoscopy findings.

In all studies, columnar epithelium was evaluated for the presence of intestinal metaplasia, inflammatory cells and intraepithelial neoplasia, which was defined according to the World Health Organization classification (160) .

3.8 IMMUNOHISTOCHEMISTRY (STUDY 1)

Specific antibodies to CD10 (56C6 Novocastra, Newcastle,UK) and Ki67 (clone K-2, Zytomed Systems, Berlin, Germany) were used as markers for differentiation and proliferation, respectively. For retrieval of antigens, deparaffinized sections were heated in citrate buffer (pH 6.0). Endogenous peroxidase was blocked by 20 min incubation with 0.3% hydrogen peroxidase in absolute methanol. Sections were washed and non-specific binding was blocked using normal serum (Nichirei, Tokyo, Japan). Overnight incubation at 4^{o}C was carried out for binding of the primary antibody. Afterwards, 30 min incubation with biotinylated secondary antibody was performed followed by substrate binding by using streptavidin-biotin-peroxidase method. Additional counterstaining with haemalaun was carried out in all cases. All stains were accompanied by negative and positive controls and only accepted if controls showed expected results. Otherwise, staining was repeated until internal controls showed appropriate results. For evaluation of the proliferation index, cells in the most affected area with positive signals against Ki67 were counted and scored along a 0–3 scale, where grade 0 = < 5%; grade 1 =5-35%; grade 2 = 36%-65%; grade 3 = > 65% of the cells stained positive (161). CD10 was semiquantitatively graded according to the Remmele Score system (162).

3.9 STATISTICS

3.9.1 Study 1

Statistical software STATA (version 11.2, Stata Corp, College Station, TX, USA) was used for data analyses. Values were expressed as median and interquartile ranges (IQR). Differences between groups 1 and 2, in HRQL and in acid reflux, were evaluated using the Wilcoxon test. Comparisons between subgroups (e.g., group1 reflux vs group 2 reflux) was conducted using Mann–Whitney U test.

3.9.2 Study 2

Statistical software SPSS (version 19.0 SPSS Inc, Chicago, IL, USA) was used for data support and analysis. Cohen's coefficient and proportion of agreement was calculated as measures of agreement between observers in the classification of endoscopic images. *k* values were estimated based on intra-class correlation coefficient (with 95% CI). Strength of agreement was considered as follows: 0 to 0.2, slight; 0.2 to 0.4, fair; 0.4 to 0.6, moderate; 0.6 to 0.8, substantial; 0.8 to 1, almost perfect. Each video classification was compared with the histological diagnosis of the corresponding specimens (gold standard). Sensitivity, specificity, and predictive values were calculated. Global accuracy was estimated based on the proportion of true-positive and true-negative results.

3.9.3 Study 3

Statistical software STATA (Version 11.2, Stata Corp, College Station, TX, USA) was used for data analyses. All analyses were conducted for dysplasia and IM, respectively, versus other diagnoses. Each video classification was compared with the histological diagnosis of the corresponding specimens (gold standard). To study the learning curve, we separately analyzed results from ME-NBI between experienced and unexperienced assessors. Hereby, we examined the outcome from three consecutive groups of videos (the first 20, the second 30, and the last 20) and also the intra-observer agreement, that is, the outcomes 1 year apart in those participating currently and 1 year earlier (study 2). At that time, the 70 videos were in the same order, but with no continuous histological feedback.

The sensitivity, specificity, global accuracy, and negative likelihood ratios (LR-) for each subgroup of observers and/or time-points of observation were computed. The LR- is computed as (1-sensitivity)/ specificity. The lower the LR-, the less likely is a patient to have the outcome under study, when having a negative result in the diagnostic test. In this context, we may consider ME-NBI useful to rule out IM or neoplasia in subjects not classified as having these outcomes by the assessors, when the LR- is below 0.2 or, preferably, below 0.1. The inter-observer agreement regarding the classification of the videos was estimated through the kappa coefficient. The results are expressed as medians and interquartile ranges. We estimated that a minimum sample size of 400 observations was required to evaluate the variation of sensitivity across the learning process, assuming an improvement of 80% to ≥ 90%, with a power of 80% and significance level of 5% and six assessors.

3.10 ETHICAL CONSIDERATIONS

The Stockholm regional ethical committee approved all the studies in this thesis. Oral and written informed consent was obtained from all patients.

4 RESULTS

4.1 STUDY 1

Fifty-eight long segment BE patients without (Group 1, n=27) or with ARS (Group 2, n=31) participated in this study. There were no significant differences in baseline characteristics between the patients in both groups (Table 2). Three patients of group 1 dropped out at baseline assessment; two due to technical problems with pH monitoring and one due to a large hiatal hernia precluding manometry, which was also the reason for one drop-out in group 2. In group 1, the final analyses were based on 24 patients (18 males, 6 females), with median age of 64.7 years (range 43-77) and median BE length of 5 cm (range 3-15). In group 2, we studied 30 patients (23 males, 7 females), with median age of 64.2 years (range 37-73) and median BE length of 5 cm (range 3-12).

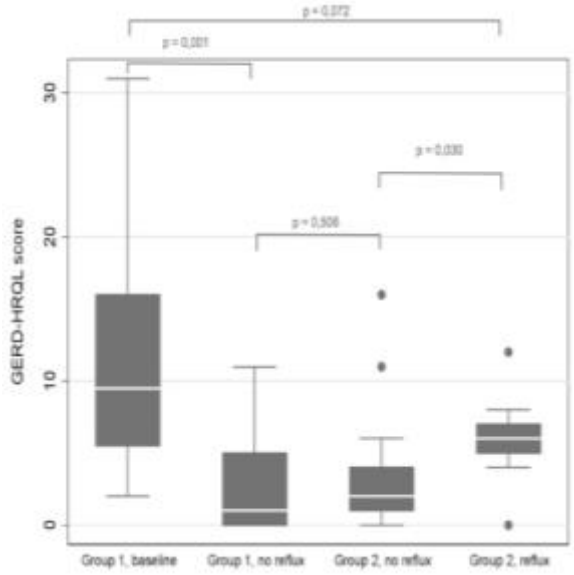
Patient characteristics	**Group 1 (n=24)**	**Group 2 (n=30)**	**p-value**
Age (years)	64.7 (56.0-67.9)	64.2 (60.0-67.6)	0.889
Gender, % men	75	77	0.887
Body mass index (kg/m^2)	27.6 (25.0-30.3)	26.2 (25.0-29.1)	0.623
Smoking, % current smokers	20.8	10.7	0.313
Barrett's esophagus length (cm)			
C - circular extent	2 (1-6)	1 (0-3)	0.099
M - maximum extent	5 (4-8)	5 (3-7)	0.278

Table 2. Demographics of patients with long segment Barrett's esophagus. Medians and 25-75 percentiles are given, unless otherwise specified.

In group 1 at baseline, a significant correlation between total acidic reflux time and both circumferential and total BE length was observed (p=0.002 and 0.003, respectively). A daily dose of 40 mg of pantoprazole normalized acid reflux in 14 of the 24 (58%) patients. Doubling the dose to 80 mg/day normalized reflux in another 2 patients, but still left 8 with abnormal acid reflux where the dose was then escalated to 120 mg/day. Among those, 3 remained unresponsive, while 1 patient did not tolerate the highest dose of pantoprazole. Three of these 4 patients finally normalized acid reflux after switching to esomeprazole 120 mg/day and bed-time ranitidine 300 mg, leaving only one patient with continued elevated esophageal acid exposure. In group 1, we observed that normalization of acid reflux was associated with a significant reduction in GERD-HRQL scores as compared to baseline values (p=0.001, Figure 7a). However, when considering each individual step of the respective dose escalation, we were able to statistically substantiate a clear difference in GERD-HRQL symptoms as a response only to the initial 8 weeks of therapy (i.e. 40 mg daily of pantoprazole, p<0.001, Figure 7b). There was no significant correlation between the different steps of PPI dose, changes in symptoms and in acid reflux, irrespective of supine or upright body positions (Figure 8).

In group 2, abnormal acid reflux with a total reflux time of 18.9% (range 7.5-27.3%) was detected in 12/30 (40%) patients; in the remaining 18 patients with a fundoplication, a total reflux time of 0.7% (range 0-4%) was recorded. Absence of pathological acidic reflux in anti-reflux operated patients was associated with significantly lower GERD-HRQL symptom scores (p= 0.030) attaining the same level as PPI-treated BE patients with normalization of acid reflux (Figure 7a).

7a



7b

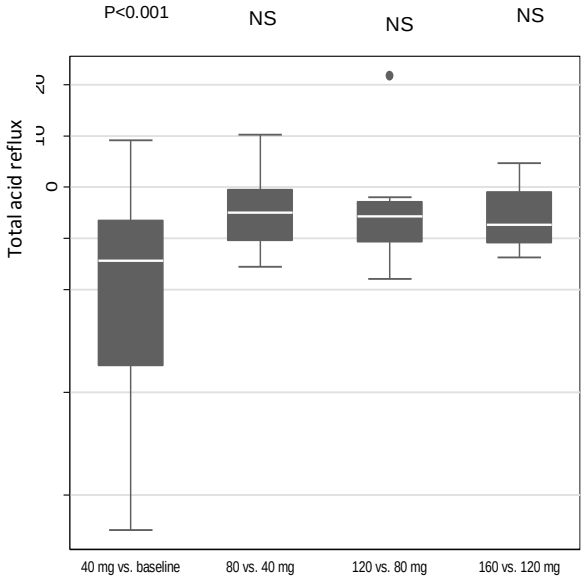
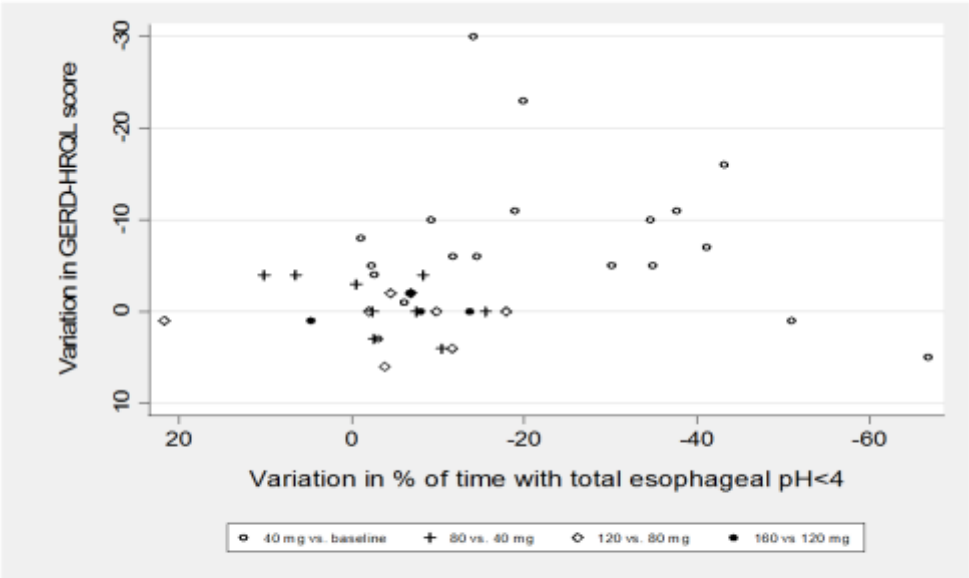


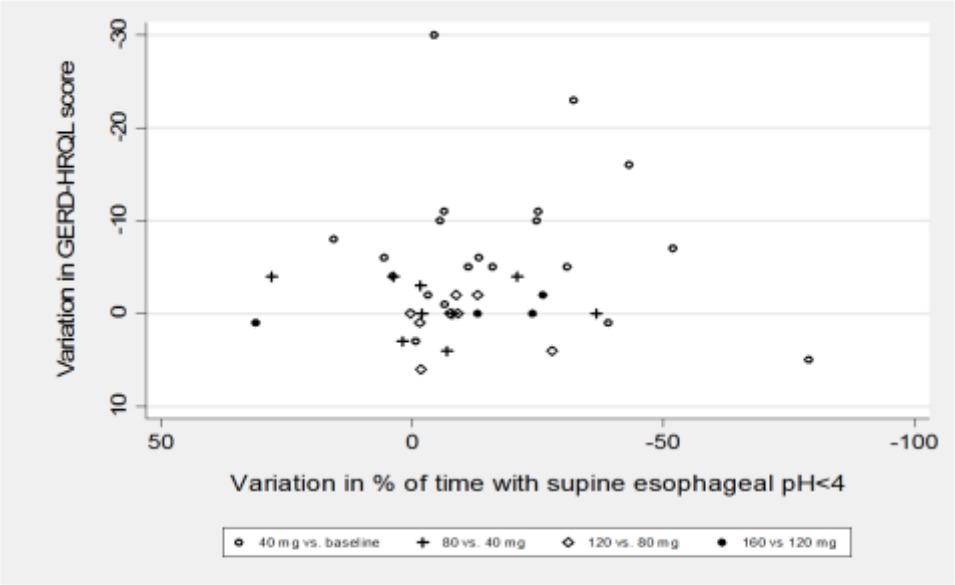
Figure 7. GERD-HRQL scores in patients in group 1 and 2, with and without acid reflux (a). Changes in total acidic reflux in group 1, as related to the different steps of the PPI escalation strategy (b). Medians, 25-75% quartiles and 10–90% ranges.

8a



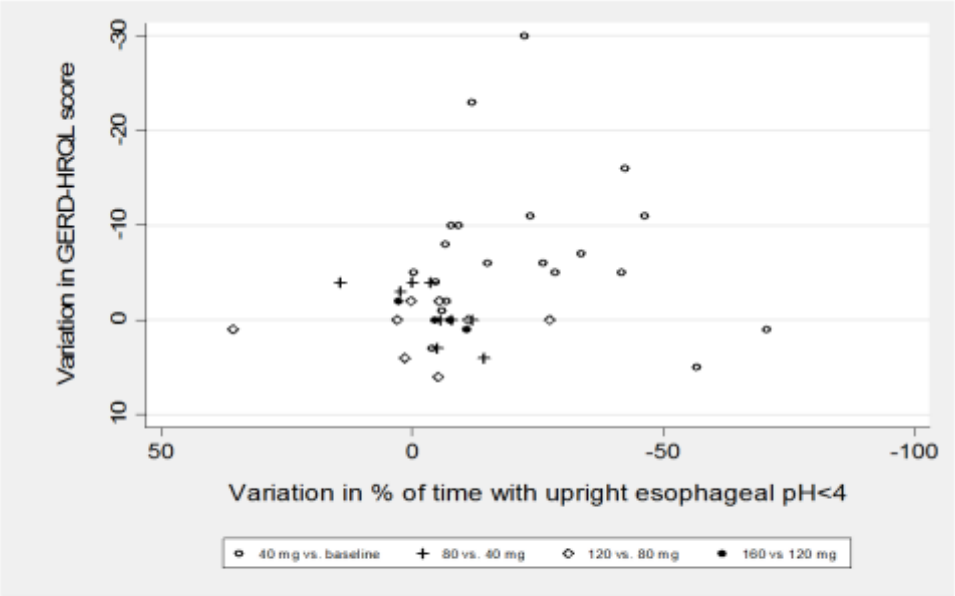
R= 0.11	R=-0.57	R=0.14	R=0.32
P=0.63	P=0.11	P=0.75	P=0.68

8b



R= 0.04	R=-0.29	R=0.20	R=0.95
P=0.86	P=0.44	P=0.64	P=0.05

8c



R= 0.04	R=-0.29	R=0.20	R=0.95
P=0.86	P=0.44	P=0.64	P=0.05

Figure 8. Correlation between the change in acid reflux variables (pH<4 during less than 4% of time) and the variation of the Gastroesophageal Reflux Disease-Health Related Quality of Life (GERD-HRQL) in relation to the different PPI titration steps in BE patients. Data are presented for the corresponding relationship to the acid reflux changes occurring in total time, and in the supine and upright positions (a, b and c respectively). R = correlation coefficient.

At baseline, established squamous epithelium markers for GERD, i.e. papillary length, basal cell layer thickness and width of intercellular spaces were all increased, as compared to published data from healthy subjects (16). Normalization of acid reflux decreased most of these variables, reaching statistical significance for intercellular spaces and papillary lengths in the squamous epithelium of group 1. In group 2, a similar picture with values towards more normal basal cell thickness was observed in those having non-pathological reflux. In the squamous, as well as in the columnar epithelium, the grading of inflammation did not change in a consistent way, neither from the distal to the more proximally located biopsy sites nor in response to therapy.

The CD10 marker of differentiation stained negative from baseline and onwards regardless of location of the tissue samples. The semiquantitative analyses of Ki67 in the columnar lined esophagus and in the squamous epithelium 1 cm above the neo-squamo-columnar junction revealed no effects in response to normalization of acid reflux parameters, irrespective of location. Moreover, we were unable to detect any differences between patients on PPI as compared to those with a previous fundoplication. In the latter group, we found no differences between those, who despite symptom control, had remaining abnormal acid reflux as compared to those in whom reflux had been completely eliminated.

4.2 **STUDY 2**

In total, the 84 evaluation videos were viewed 3 times by each of the 9 assessors, corresponding to a total of 2268 video clips reviewed and rated. The median evaluation time for all videos and classification systems was 25 seconds (IQR 20-39 seconds). For the Amsterdam system, significantly more time was needed (median 29 seconds, IQR 23-45 seconds; $p < 0.001$, Kruskal-Wallis test). There was no significant difference in evaluation times in relation to the level of the assessors' expertise.

For further assessment of video quality and classification feasibility, assessors notified whether each video was suitable for mucosal morphology evaluation. The videos were rated as unclassifiable regarding mucosal and vascular patterns, respectively, in 40 (5.2%) and 6 (0.8%) cases when using the Kansas classification, in 14 (1.8%) and 3 (0.4%) cases when using the Amsterdam system, and in 1 (0.1%) and 5 (0.7%) cases when using the Nottingham classification. The raters were also asked to indicate the level of certainty in the histological prediction. The overall certainty was significantly higher in the non-expert group ($p < 0.005$, χ^2), irrespective of the classification system assessed. We were unable to demonstrate any difference among the 3 classification systems ($p = 0.468$, χ^2) in this regard.

Sensitivity and specificity for the detection of non-dysplastic IM were 37% and 69% for the Kansas, 53% and 68% for the Amsterdam, and 43% and 65% for the Nottingham systems, respectively. All classification systems showed better sensitivity and specificity for dysplastic IM; i.e. 78% and 74% for Kansas, 81% and 71% for Amsterdam, and 73% and 75% for Nottingham systems, respectively. There was no significant difference in the detection of nondysplastic and dysplastic IM as related to the observers' level of expertise (Table 3).Global accuracy was 47% for the Kansas, 51% for the Amsterdam, and 46% for the Nottingham classification systems, respectively (difference not statistically significant). There was a positive association between the grading of the histology and the accuracy of the endoscopic prediction. No significant impact was observed related to the level of the assessors' expertise (Table 4).

The overall inter-observer agreement was "moderate" for the Kansas and Amsterdam classification systems with global κ values of 0.44 (95% CI, 0.35-0.55) and 0.47 (95% CI, 0.38-0.56), respectively, but only fair for the Nottingham classification system (κ = 0,34; 95% CI, 0.26-0.43). The respective observer's level of expertise had no influence in the outcome.

Classification System	Observers	Nondysplastic IM		Dysplastic IM	
		Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Kansas	Experts	0.38 (0.31-0.44)	0.68 (0.62-0.73)	0.73 (0.68-0.79)	0.79 (0.74-0.84)
	Experienced assessors	0.36 (0.30-0.42)	0.71 (0.65-0.76)	0.82 (0.78-0.87)	0.75 (0.69-0.80)
	Unexperienced assessors	0.39 (0.33-0.45)	0.70 (0.64-0.76)	0.78 (0.72-0.83)	0.70 (0.64-0.75)
	Global	0.37 (0.31-0.43)	0.69 (0.64-0.75)	0.78 (0.73-0.83)	0.74 (0.69-0.80)
Amsterdam	Experts	0.58 (0.51-0.64)	0.56 (0.50-0.63)	0.68 (0.62-0.74)	0.83 (0.78-0.88)
	Experienced fellows	0.54 (0.48-0.60)	0.72 (0.66-0.78)	0.90 (0.86-0.94)	0.70 (0.64-0.76)
	Nonexperienced fellows	0.48 (0.42-0.55)	0.77 (0.71-0.82)	0.86 (0.82-0.91)	0.61 (0.54-0.67)
	Global	0.53 (0.47-0.60)	0.68 (0.62-0.74)	0.81 (0.77-0.86)	0.71 (0.66-0.77)
Nottingham	Experts	0.42 (0.36-0.49)	0.58 (0.52-0.64)	0.60 (0.54-0.67)	0.81 (0.76-0.86)
	Experienced assessors	0.44 (0.37-0.50)	0.66 (0.60-0.72)	0.80 (0.75-0.85)	0.75 (0.69-0.80)
	Unnexperienced assessors	0.42 (0.36-0.49)	0.71 (0.65-0.77)	0.79 (0.74-0.84)	0.69 (0.64-0.75)
	Global	0.43 (0.37-0.49)	0.65 (0.59-0.71)	0.73 (0.68-0.79)	0.75 (0.70-0.81)

Table 3. Sensitivity and specificity for detection of non-dysplastic and dysplastic specialized intestinal metaplasia using different systems for Barrett's esophagus classification with magnification endoscopy and narrow-band imaging (CI, Confidence interval; IM, intestinal metaplasia).

Classification System	Observers	Accuracy for nondysplastic IM (95% CI)	Accuracy for dysplastic IM (95% CI)	Global accuracy (95% CI)
Kansas	Experts	0.57 (0.51-0.63)	0.77 (0.72-0.82)	0.47 (0.40-0.53)
	Experienced assessors	0.58 (0.52-0.64)	0.77 (0.72-0.83)	0.49 (0.43-0.60)
	Unexperienced assessors	0.58 (0.52-0.64)	0.72 (0.67-0.78)	0.46 (0.40-0.56)
	Global	0.57 (0.52-0.64)	0.75 (0.70-0.81)	0.47 (0.41-0.53)
Amsterdam	Experts	0.57 (0.51-0.63)	0.78 (0.73-0.83)	0.50 (0.43-0.56)
	Experienced assessors	0.66 (0.60-0.72)	0.77 (0.71-0.82)	0.54 (0.48-0.60)
	Unexperienced assessors	0.67 (0.61-0.79)	0.69 (0.63-0.75)	0.50 (0.44-0.56)
	Global	0.63 (0.57-0.69)	0.75 (0.65-0.80)	0.51 (0.45-0.57)
Nottingham	Experts	0.53 (0.46-0.59)	0.75 (0.69-0.80)	0.43 (0.37-0.50)
	Experienced assessors	0.58 (0.52-0.65)	0.77 (0.71-0.82)	0.48 (0.41-0.54)
	Unexperienced assessors	0.61 (0.55-0.67)	0.73 (0.67-0.73)	0.48 (0.42-0.54)
	Global	0.57 (0.51-0.64)	0.75 (0.68-0.81)	0.46 (0.40-0.53)

Table 4. Diagnostic accuracy for detection of nondysplastic and dysplastic intestinal metaplasia using different systems for Barrett's esophagus classification with magnification endoscopy and narrow-band imaging (CI, Confidence interval; IM, intestinal metaplasia).

4.3 **STUDY 3**

Each of the six observers completed the assessment of all 70 videos, corresponding to a total of 420 videos observed and rated. During the learning process, there was a significant decrease in the time needed for each video evaluation, both among experienced ($p = 0.002$) and unexperienced endoscopists ($p = 0.001$). By and large the experienced endoscopists used shorter time for evaluation than those under training ($p < 0.001$). Moreover, within the experienced group, less time was required during the present evaluation, compared to the evaluation completed 1 year earlier ($p < 0.001$).

As seen in Figure 8, a substantial difference was observed in the certainty by which assessors scored the histological prediction, a difference, which was highly dependent on the level of expertise ($p < 0.001$). However, within the learning process, no significant changes were observed, neither among experienced nor among unexperienced assessors. Within the experienced group that had made an evaluation 1 year earlier, the later assessment was completed with a higher level of certainty ($p = 0.016$).

Considering the experienced observers, the median (range) sensitivity and specificity for detection of IM was 44% (33–57) and 79% (71–85), respectively. The corresponding figures for neoplasia were 84% (73–92) and 76% (68–83). In the group of unexperienced observers, the median (range) sensitivity and specificity for detection of IM was 47% (35–59) and 72% (64–80), respectively, whereas the corresponding figures for neoplasia were 75% (62–85) and 76% (68–82). The global accuracy ranged from 56% to 77% for IM and from 70% to 85% for neoplasia. The negative likelihood ratio ranged from 0.49 to 0.93 and from 0.12 to 0.52 for IM and neoplasia, respectively. No significant differences were seen between NBI-experienced endoscopists and those under training, nor could we demonstrate any effect of the learning process.

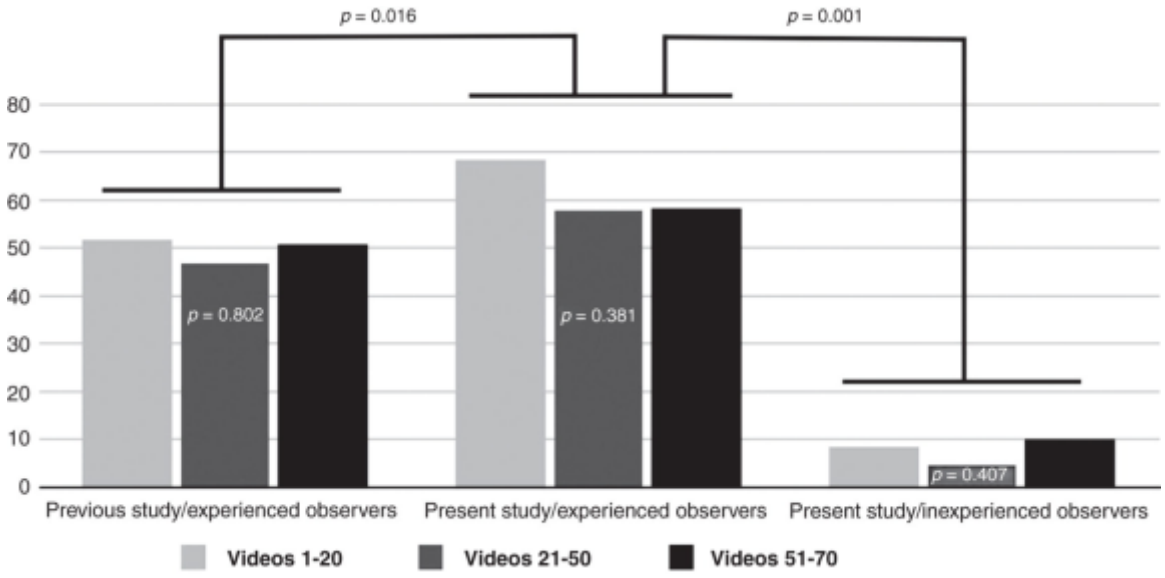


Figure 8. Scored levels of certainty on the histological prediction using the Amsterdam classification among experienced and unexperienced observers. For experienced observers, data from the study 3 as well as from our previous study (Study 2) are displayed.

The overall inter-observer agreement was generally low, ranging from 0.25 to 0.30 for IM and from 0.39 to 0.48 for neoplasia. There were no significant differences relating to the level

of the assessors' experience (Table 5). The intra-observer outcome, when the assessment was repeated with one year's interval, revealed basically the same figures (Table 6).

		Inter-observer agreement (95% confidence interval)			
Videos		**1–20**	**21–50**	**51–70**	**All videos**
Nonneoplastic IM	Experienced assessors				
	Previous study (Study2)	0.58 (0.32–0.83)	0.16 (0.00–0.37)	0.28 (0.03–0.53)	0.32 (0.45–0.18)
	Present study (Study3)	0.27 (0.01–0.52)	0.11 (0.00–0.32)	0.44 (0.19–0.69)	0.25 (0.12–0.39)
	Unexperienced assessors	0.42 (0.17–0.68)	0.23 (0.03–0.44)	0.28 (0.03–0.53)	0.30 (0.17–0.44)
Neoplastic IM	Experienced assessors				
	Previous study (Study2)	0.87 (0.61–1.00)	0.51 (0.30–0.71)	0.73 (0.48–0.98)	0.67 (0.54–0.81)
	Present study (Study3)	0.26 (0.01–0.52)	0.39 (0.18–0.60)	0.52 (0.27–0.77)	0.39 (0.26–0.53)
	Unexperienced assessors	0.60 (0.35–0.85)	0.42 (0.21–0.62)	0.41 (0.16–0.67)	0.48 (0.35–0.62)

Table 5. Inter-observer agreement for the Amsterdam classification system stratified by the level of experience and final histological grade. Data from previous study (Study 2) versus present study (Study 3) is used.

	Intra-observer agreement (95% confidence interval)		
	Observer 4	**Observer 5**	**Observer 6**
Nonneoplastic SIM	0.32 (0.10–0.55)	0.41 (0.18–0.65)	0.26 (0.03–0.49)
Neoplastic SIM	0.51 (0.28–0.74)	0.82 (0.59–1.00)	0.52 (0.29–0.75)
All diagnoses	0.38 (0.21–0.54)	0.56 (0.39–0.73)	0.34 (0.19–0.50)

Table 6. Intra-observer agreement of the experienced observers using the Amsterdam classification system. Data from previous (Study 2) versus present study (Study 3) is used.

5 GENERAL DISCUSSION

The first part of this thesis evaluated the impact of increased doses of PPI in long segment BE, namely on esophageal acidic reflux, health related quality of life, and histology. We then compared the results in this cohort with a cohort of patients with long segment BE with previous clinically successfully ARS. Our study is innovative as it uses stepwise increases of PPI and compares these 2 different strategies for BE management.

In the second part of this thesis we evaluated the role of NBI in BE characterization. These studies are innovative since we used videos instead of still pictures for mucosal assessment, evaluated different NBI classification systems, introduced the concept of certainty on prediction in this context, and used a computerized learning process with systematic feedback. Some of these features were applied in subsequent publications on enhanced endoscopy (81, 163).

5.1 PROTON PUMP INIHIBITORS

PPI are effective acid suppressive drugs and the most common used drugs in the management of GERD disorders. They are considered safe but may differ in efficacy, interactions and safety profile. PPIs do have some limitations related to their short plasma half-lives and requirement for meal-associated dosing (164).

Esophageal acid reflux is considered a major factor for BE formation and in its neoplastic progression. While all current guidelines propose the use of PPI in the treatment of esophagitis, GERD-related symptoms or after ablation therapy in BE, their use for chemoprevention is still controversial. In fact, only one of current guidelines advocates PPI use for this purpose (17).

5.1.1 PPI in symptoms control

GERD-related symptoms are present in 80% of long segment BE and in 45% of short segment BE patients. As there is some heterogeneity among studies regarding GERD symptoms assessment, we used a validated GERD-HRQL score that evaluates patient's perception of symptoms severity. In our study with long segment BE patients, the first step with 40 mg pantoprazole once daily, was associated with a significant reduction in GERD-HRQL scores. Increased doses in patients with acidic reflux were not associated with significant improvement in symptoms. This impact of standard PPI dosing in symptoms relief regardless of acid suppression has been described before (165).

Considering our results and previously published data, it may be assumed that the goal of symptoms relief can be achieved in most patients with standard PPI dose, even in long-segment BE. Nevertheless, depending on the alleged reduced sensitivity of the esophageal mucosa in BE, it can be argued that significant clinical improvements are achieved already as a response to the initial changes in acid reflux that still might be far from normalization.

5.1.2 PPI and control of acidic reflux

Control of acidic reflux is more difficult to be achieved in BE patients than in GERD patients

without BE (164). That is especially true in long segment BE, mainly due to poor anti-reflux mechanisms. Several studies had evaluated the suppression of acid reflux using standard or high doses of PPIs (165, 166). In most of them, it was not possible to achieve acid suppression in a significant number of patients. Therapeutic failure, may occur in rapid PPI metabolizers who have less available drug at a given dose. In contrary, poor metabolizers may be at risk for over-treatment, with increased incidence of adverse effects and unnecessary costs. A solution to this problem may be phenotyping or, preferably, genotyping patients prior to treatment with PPIs. This would enable tailoring dose regimens according to individual metabolic profile. An alternative strategy is the development of PPIs that are either metabolized by genotype-independent mechanisms or are less susceptible to inter-individual genetic variation (166).

Contrary to previous studies, we did not use pre-defined PPI doses, but a step-wise dose increase, until pH normalization. We started with the standard, once daily dose approved for GERD, and a stepwise approach aiming to address the daily practice as expressed by current guidelines (17). In our study, with the initial dose of 40 mg once daily, acid suppression was achieved in 58% (14/24) of patients. That may be related to PPI characteristic, with short plasma half-lives that lead to breakthrough in acidic reflux and symptoms in some patients. Our results support the results from previous studies, namely one with 30 BE patients, showing the presence of pathological reflux in 40% of patients with PPI once daily, despite normalization of symptoms (163). In our study, it was possible to obtain acid suppression in all but one patient, but 42% of patients needed higher PPI doses. Our findings support also a recent report with 23 patients, showing a 90% acid reflux suppression on high doses of PPI twice daily (167). Similar results were also described in previous studies with PPI twice daily (161, 168). Considering our results and published literature, it may be expected that in the majority of long segment BE patients, complete acid suppression may only be achieved with high PPI doses, at least double dose regimens. However, it is unknown if all BE would benefit from full acid suppression. It has been demonstrated that such strategy is necessary for restitution of normal squamous epithelium after Barrett ablation therapy, being poor acid suppression associated with poor response to ablation (169, 170). So, proper acid suppression with at least double dose of PPI shall be considered in patients planned for endoscopic treatment. Probably new PPI formulas with longer plasma half-lives or extended release drugs may increase the efficacy of PPI drugs in acid suppression (171, 172).

5.1.3 PPI and histology

While acid suppression after ablation treatment aims to the restitution of new squamous epithelium, the potential role of acid suppression in non-dysplastic BE is to avoid or delay progression into neoplastic BE. Histology, namely the grade of dysplasia, continues to be the only accepted marker for risk stratification in BE. Non-dysplastic BE constitutes the largest proportion of BE patients and there is still controversy on the preventive use of PPI in this group of patients (137, 141, 142). PPI are considered safe and their costs have decreased dramatically, but the risk of neoplastic progression of BE in these patients is low. Current BE guidelines do not recommend more than one daily dose of PPI for prevention of BE progression (17, 47). It is however not known if an increase to double dose and the resulting improvement in acid suppression would have relevant clinical impact in those BE patients. As the major pool of BE patients has no dysplasia at first endoscopy and has a low risk of

neoplastic progression, the use of appropriate biomarkers would enable selection of those patients that would benefit from surveillance or therapy (173). Changes in biomarkers' levels could also assist in the monitoring of therapies such as PPI in BE. Until now, there are no validated biomarkers for clinical use, besides conventional histomorphology.

Acid reflux is considered the main trigger for cellular differentiation and proliferation in Barrett's mucosa. In order to test the impact of acid suppression on BE mucosa, we evaluated acute and chronic inflammatory parameters, and markers of cellular proliferation and differentiation (Ki67 and C10, respectively) in Barrett's and squamous epithelium according to the levels of acid suppression. We also evaluated the morphological changes at the distal squamous epithelium, according to acid reflux levels. Changes in acute and chronic inflammation markers did not display a consistent pattern related to the control of acid reflux. Markers for the proliferative drive on the columnar lined, as well as squamous epithelium, were outside the normal ranges (159), but importantly, these parameters remained stable and unaffected either by up-titration of PPI doses or fundoplication. Contrary to others, we did not find any significant difference in cell proliferation marker Ki67 before PPI treatment and after full acid suppression (161). That may be related to the fact that most patients were on full anti-secretory therapy for only 8-16 weeks and probably a longer period would be necessary to achieve effects on cellular proliferation (161).

Other markers of reflux-induced damage to the squamous epithelium are represented by the papillary length, basal cell layer thickness and the width of the intercellular spaces. These variables had not been studied previously in the most distal squamous epithelium of long-segment BE patients. We observed only a marginal effect of therapy in the direction towards normalization, but these changes are different from what has been demonstrated to occur in response to PPI therapy in the distal esophagus of GERD patients (174). It might be argued that baseline data were captured after a too limited washout period of time for duodeno-gastro-esophageal reflux to exert its full damaging effect. However, basically all similar studies have applied a corresponding or even shorter washout period (167, 175, 176). Since even short acid pulses can stress the Barrett' s mucosa in an unfavorable direction, our results would offer a background for the use of a tailored strategy in high risk BE individuals.

5.2 ANTI-REFLUX SURGERY

As well as treatment with PPIs, ARS is effective in controlling acid reflux in BE patients. However, unlike PPIs, ARS may suppress all esophageal reflux including non-acidic reflux that may promote BE formation and neoplastic progression.

5.2.1 ARS and symptoms

Few studies had previously evaluated the effect of ARS on symptoms in BE (154, 177). Most of these studies used neither detailed nor validated assessment tools. In this context, it is pertinent to bring into focus the observation done in our BE patients with a fundoplication. Although all included patients considered themselves as symptom-free on a telephone interview and devoid of any requirements for anti-secretory drug therapies, a significant number of them still displayed GERD-HRQL related symptoms. This illustrates the importance of adding objective means to determine the efficacy and durability of GERD

control after surgical repair, especially in BE. Another relevant finding in our study is that the level of GERD-HRQL is similar after full acid suppression on PPI and successful ARS surgery. So, one should expect same symptoms control under optimal medical or surgical therapy in long segment BE.

5.2.2 ARS and acidic reflux

Contrary to PPIs, ARS aims to correct the failure of lower esophageal sphincter and to repair the frequent hiatal hernia in patients with long segment BE. In our operated patients, absence of pathological acidic reflux was associated with significantly lower GERD-HRQL symptom scores ($p = 0.03$). It is relevant to notice that abnormal acidic reflux with a total reflux time of 18.9% (range 7.5-27.3%) was detected in 12/30 (40%) of our operated patients. The presence of BE has been described as a strong risk factor for failure of ARS (177, 178), and recent data suggests that this procedure is more demanding in the presence of BE (179). In our cohort of operated BE patients, ARS was performed more than 5 years before inclusion and this may also have contributed to the high number of patients with pathological reflux. It was recently demonstrated in a Swedish population-based study on GERD patients with ARS that recurrent reflux is substantially more common among patients that subsequently develop esophageal adenocarcinoma than among those who do not develop it (180). From a clinical perspective, our findings and published literature suggest that results of ARS should be evaluated carefully, and that patients with long segment BE with fundoplication and persisting reflux should be considered for a detailed surveillance protocol.

5.2.3 ARS and histology

There are theoretical aspects and experimental data to support the notion that complete reflux control would be preferable to reach the environmental condition that would minimize the mucosal stress and the proliferative drive towards neoplastic transformation. Concerning histological results, we did not find significant differences neither between chronic or acute inflammation nor between proliferation or differentiation markers parameters between ARS patients with or without pathological acid reflux.

In both long segment BE groups (PPI and ARS), we detected a persistence of dilated intercellular space in the distal squamous epithelium, irrespective of group or reflux status. That may reflect a phenotypic characteristic of BE or at least long segment BE that was not described before. Dilated intercellular space has been classically been associated with acidic reflux and more recently with biliary reflux, and may be associated with non-erosive reflux disease (NERD) symptoms, namely in patients lacking symptomatic improvement during PPI therapy. There are no published studies specifically evaluating dilated intercellular space in BE under PPI therapy. Two related studies in GERD that included BE patients had smaller sample of BE patients (181, 182) and PPI therapy was excluded. Thus, in these studies the increased dilated intercellular space could be related to increased acid reflux. Our finding of dilated intercellular space in 2 different cohorts of long segment BE irrespective of reflux status is new and may lead to further studies in the field.

5.3 NARROW BAND IMAGING

New endoscopic imaging technologies have been developed in the last years, aiming to improve visualization of the mucosa along the gastrointestinal tract. In BE, these techniques intend to enhance detection or improve characterization of lesions. Enhanced detection technologies aim to act as red flag tools in the identification of lesions that may harbor early neoplasia. They are used during broad field overview endoscopy, mainly in surveillance endoscopy, and their ultimate goal is to replace random biopsies that are time and money consuming. Enhanced characterization technologies are usually focused in the evaluation of small mucosal areas using magnification. They aim to evaluate detected lesions, in order to differentiate early neoplasia from non-neoplasia. This is pivotal in BE because early neoplastic lesions are suitable for curative endoscopic treatment.

Barrett's mucosa is characterized by its mosaic structure, with different types of epithelium. That, combined with the fact that neoplastic tissue may have different grades of dysplasia, turns mucosal assessment difficult. Endoscopic techniques for detection and characterization of lesions in BE must be user-friendly and accurate, before their use can be disseminated. Several studies describe the used of high-definition endoscopy and virtual chromoendoscopy aiming to address these goals. The first studies came from different centers, leading to the proposal of different classification systems. However, all these studies used still pictures that do not resemble daily endoscopy practice, and sometimes used capture and selection methodologies that are not well characterized. In addition, several of these studies used a high ratio of neoplastic vs non-neoplastic pictures that does not resemble clinical practice and thus can induce selection bias.

In our second study, we evaluated classification systems proposed by 3 different groups: Kansas, Amsterdam and Nottingham. We used randomly selected videos representing the practice in a tertiary hospital. The 3 classification systems were found to be useful in Barrett's mucosa assessment, but all showed limitations in accuracy for identification of intestinal metaplasia and dysplasia, with suboptimal inter-observer agreement. As these are new technologies, and as the Amsterdam classification system was the one with better outcome, we performed the study 3 aiming to evaluate if a dedicated learning program could improve accuracy in BE assessment. We concluded that the Amsterdam classification system remains suboptimal in terms of accuracy and inter- and intra-observer agreement, even after a detailed learning process empowered by continuous feedback. According to our results, random biopsies following the Seattle protocol and biopsies of all detection lesions are still mandatory in clinical practice. That was also confirmed by following studies.

Like in most studies in the field (81), studies 2 and 3 used per-area and not a per-patient assessment, which may induce a selection bias, as only some areas of all BE were evaluated and only the best quality videos were selected for posterior evaluation. Other studies had used per-patient evaluation of BE comparing different strategies (133, 183). But irrespective of per-area or per-patient approach, most of studies did not show superiority of targeted biopsies compared with random biopsies. Also, most of these studies were underpowered for the detection of dysplasia. That may hamper the use of different classification systems in current clinical practice. In addition, considering that even conventional histomorphological assessment may become difficult, namely for the diagnosis of LGD (184), it should be expected that also high definition magnification endoscopy has limitations in the identification of LGD or in the differentiation between columnar epithelium with or without IM (163). Another important fact to consider when interpreting studies on endoscopic characterization of BE is that all videos or pictures are assessed against the histological result obtained by conventional biopsy sampling. Several studies had shown that areas/lesions in

BE previously characterized by biopsy sampling were down- or up-staged after endoscopic mucosal resection in up to 30% of cases (185), being mucosal resection also associated with an increase in inter-observer agreement among pathologists (186). Considering the published literature, their results and limitations, current technologies cannot as yet replace random biopsies and targeted biopsies of visible lesions in common clinical practice (187). In the future, developments with automatic and real-time endoscopic assessment or with molecular biomarkers added to image enhanced endoscopic would change the current practice (188).

6 CONCLUSIONS

Referring to the described aims of the study, the following conclusions can be formulated:

1. Intraesophageal acid reflux variables co-varied with symptom scores in patients with long-segment BE, throughout the upwards titration of PPI doses. We observed an association between the degree of symptom relief and the change in acid reflux variables and it was possible to normalize acid reflux in long-segment BE patients, based on the principle of step-wise increasing doses of the PPI, adjusted to the remaining reflux patterns detected during ambulatory 24-hour pH monitoring.

2. Tailored medical therapy can reach the same level of reflux and symptom control as a clinically successful fundoplication. There seems to be no difference in symptom profiles between these two patient groups.

3. Changes in acute and chronic inflammation markers did not display a consistent pattern with the control of acid reflux, and no differences were found between those given PPI and those submitted to clinically successful ARS. However, an improvement was recorded in the squamous epithelium in most parameters alleged to represent reflux-induced damage. We described far more discrete changes in response to therapy than previously observed in the distal esophagus of GERD patients without BE. Markers for the proliferative drive on the columnar lined, as well as squamous epithelium, were outside the normal ranges, but these parameters remained stable and unaffected either by up-titration of PPI doses or by presence of a well-functioning anti-reflux valve.

4. All the available NBI classification systems could be used in a clinical environment, but with inadequate inter-observer agreement. All classification systems based on combined ME and NBI revealed substantial limitations in predicting nondysplastic and dysplastic BE, when assessed externally. Thus, this technique cannot as yet replace random biopsies for histopathological analysis.

5. Using a dedicated learning program, the ME-NBI Amsterdam classification system remains suboptimal in terms of accuracy and inter- and intra-observer agreements. These results reiterate the questionable utility of corresponding classification system in clinical routine practice.

7 POPULÄRVETENSKAPLIG SAMMAFATTNING

Barrets esophagus anses uppstå som en följd av långvarig svår gastroesofageal reflux med en motsvarande kraftig irritation of inflammation som slemhinnan i distala esofagus utsätts för. Denna esxponering leder till att det flerskiktade skivepeitelet som normalt bekläder matstrupen omvandlas till ett s.k. körtelepitel. Detta körtelepitel kan sedan ytterligare förändras till att anta bl.a en mer intestinal-tunntarms liknande utseende. Om dessa processer för fortgå uppstår en klart ökad risk att utveckla förstadier till tumörer liksom även etablöerad invasiv körtelcancer. Den kliniska handläggningen ab BE patienter inriktar sig därför på två huvudinriktningar; dels att kontrollera refluxen, dels att upptäcka och fortsatt handlägga förestadier till körtelcancer i den omvandlade körtelslemhinnan.

Följande frågeställningar har belysts i den aktuella avhandlingen:

1. Samvarierar förekomsten av sura reflux mätt med ambulatorisk pH registreraing med symtom registrering hos LSBE patienter genom en successiv upptitrering av PPI doseringen? Går det att med denna PPI baserade strategi eliminera sura uppstötningar hos dessa patienter?

2. Kan man med PPI terapi uppnå samma nivå av normalisering av sur reflux och symtomkontroll som efter en kliniskt framgångsrik fundoplikation hos patienter med LSBE?.

3. Samvarierar förändringarna i den sura refluxen i respektive grupp med morfologiska förändringar i såväl körtel- som skivepitel?

4. Endoskopiska tekniker har utvecklats för att förbättra diagnostiken av s.k. förstadier till liksom etablerad malignitet i BE. Vad blir utfallet om man jämför olika NBI klassificeringssystem för endoskopisk bedömning av BE?

5. För att validera ytterligare ett av dessa system dvs Amsterdam NBI klassificeringen undersökte vi om ett strukturerat undervisningsprogram kunde förbättra detta klassifiseringssystem ytterligare

Patienter och metoder
Två kohorter av långa segmentet patienter studerades. En grupp (n = 24), behandlades ökande doser av PPI, i 8-veckors intervall, till dess den sura refluxen normaliserades . Innan behandlingens start och efter varje dos, gjordes ambulatorisk 24h pH mätning, endoskopi med biopsier och symtom scoring (gastroesofageal refluxsjukdom hälsorelaterad livskvalitet, GERD / HRLQ). Grupp nr 2 (n = 30) bestod av patienter med en tidigare (> 5år) genomått en fundoplikation.

I studie 2 bestod utvärderingsmaterialet av 15 filmer, vilka innehöll motsvarande gastric typ slemhinna (n = 5), nondysplastisk IM (n = 5), LGD (n = 2), och HGD / EAC (n = 3) sllemhinnor. Tre olika DVD skapades, en för varje klassificeringssystemet . Varje DVD bestod av en utbildning och en utvärdering set. För att undvika slumpmässig påverkan visades samma 84 filmer i en slumpmässig och helt ny ordning för varje DVD. Filmerna märktes på olika sätt och skickas till observatörerna vid 3 veckors intervall i slumpmässig ordning. I studie 3, valde vi de första 70 filmer från 84 slumpmässigt utvalda från studie 2, motsvarande 26 till gastric typ slemhinna, 23 till nondysplastic IM, och 21 till dysplastiska IM. Deltagarna fick först studera en pedagogisk uppsättningen av endoskopiska registreringar. Detta pedagogiska material bestod av en PowerPoint-presentation med en beskrivning av Amsterdam klassificering, en video förklaring av programvaran, följt av en serie av 15

undervisnings videoklipp. Varje deltagare kunde studera varje video så många gånger som behövs. Efter klassificeringen av varje video, bedömde varje endoskopist respektive histologi i en av följande kategorier: gastric typ slemhinna, IM, och dysplastiska BE. Vid varje tillfälle fick bedömaren ange om vederbörande var säker eller inte på den histologiska förutsägelsen. Därefter gavs histologiska återkoppling automatiskt varpå tillgång till denna video blockerades. Samma förfarande följdes för var och en av de 15 inlärnings videor och i var och en av de s.k. 70 utvärderings video klippen

Resultat

I grupp 1, normaliserades den sura refluxen hos 23 av 24 patienter sura uppstötningar, vilket resulterar i förbättrade GERD / HRQL poäng ($p = 0,001$), mest uttalade efter den initiala start dosen av PPI ($p <0,001$). PPI behandling nådde samma nivå av GERD / HRQL poängen som sågs hos de patienter som tidigare genomgått en kliniskt framgångsrik fundoplikations operation ($p = 0,5$). Normalisering av den sura reflux i båda grupperna var associerad med reduktion i papillär längd, basalcellsskikttjocklek , intercellulära utrymmena dilatation, akut och kronisk inflammation i skivepitel.

Sensitivitet och specificitet för detektion av icke-dysplastisk IM var 37% och 69% för Kansas, 53% och 68% för Amsterdam, och 43% och 65% för Nottingham systemen. Alla klassificeringssystem visade bättre känslighet och specificitet för dysplastiska IM; dvs 78% respektive 74% för Kansas, 81% och 71% för Amsterdam, och 73% och 75% för Nottingham. Det fanns ingen signifikant skillnad i detektering av nondysplasticsk och dysplastisk IM i relation till observatörerna "erfarenhetsnivå". Global tillförlitlighet var 47% för Kansas, 51% för Amsterdam, och 46% för Nottingham klassificeringssystemen (ej signifikant). Det fanns ett positivt samband mellan graderingen av histologi och presitionen i den endoskopisk förutsägelsen.

I studie 3 noterades en väsentlig skillnad i säkerheten genom vilken bedömare gjorde den histologiska förutsägelse, en skillnad som var starkt beroende av kompetens nivån ($p <0,001$). Det bör observeras att som ett utfall av inlärningsprocessen observerades inga signifikanta förändringar, varken bland erfarna eller bland oerfarna bedömare. Inom den erfarne gruppen, som hade gjort en utvärdering ett år tidigare, var den senare bedömningen förenad med en högre nivå av säkerhet ($p = 0,016$). Den övergripande överenstämmelsem mellan endoskopister var generellt låg, allt från 0,25 till 0,30 för IM och 0,39-0,48 för neoplasi. Vi fann inga signifikanta skillnader när det gäller nivån av bedömarna erfarenhet . Resultateten inom en och samma endoskopist dvs när bedömningen upprepades med ett års intervall visade i stort sett samma resultat.

Slutsatser

Denna studie visar att, hos patienter med LSBE, sur reflux och symptompoäng samvarierar genom ett flertal steg av PPIs behandlingsprocess och når samma nivå som efter en lyckad fundoplikations operation. Mindre förändringar återfanns bland GERD markörer på morfologiska nivå såväl i körtel som i skivepitelet, oavsett medicinsk eller kirurgisk behandling.

Alla av de tillgängliga systemen för BE klassificering är förenade med otillräcklig interobserver variabilitet. Alla klassificeringssystem baserat på kombinerad ME och NBI, avslöjade betydande begränsningar i att förutsäga nondysplastic och dysplastiska lesioner inom det metaplastiska epitelet. Denna teknik kan ännu inte ersätta slumpmässiga biopsier för histopatologisk analys. Med hjälp av ett särskilt utbildnings program, applicerat på ME-NBI Amsterdam klassificeringssystem suboptimal när det gäller precision och minimera inter och intraobserver variabiliteten, visar resultaten den tvivelaktiga nyttan av motsvarande klassificeringssystemet i klinisk rutin.

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