SOME NEW CLINICAL AND EXPERIMENTAL ASPECTS OF THE ETIOLOGY AND TREATMENT OF ESOPHAGEAL DISEASES

Ph.D. thesis

by

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ABBREVIATIONS

ACIP	Advisory Committee on Immunization Practices
CISH	Chromogenic In Situ Hybridization
CRT	Chemoradiotherapy
CT	Computer Tomography
DES	Diffuse/Distal Esophageal Spasm
ESCC	Esophageal Squamous Cell Carcinoma
GEA	Gastroepiploic Artery
GERD	Gastroesophageal Reflux Disease
GHRH	Growth Hormone-Releasing Hormone
GHRH-R	Growth Hormone-Releasing Hormone Receptor
IGF-I	Insuline-like Growth Factor I
HLES	Hypertensive Lower Esophageal Sphincter
HPV	Human Papillomavirus
Hsp	Heat Shock Protein
HUES	Hypertensive Upper Esophageal Sphincter
iSTS	idiopathic Subglottic Tracheal Stenosis
LES	Lower Esophageal Sphincter
LTS	Laryngotracheal Stenosis
PPI	Proton Pump Inhibitor
RECIST	Response Evaluation Criteria in Solid Tumors
SCE	Specialized Columnar Epithelium
SqE	Squamous Epithelium
SR	Schatzki's ring
STS	Subglottic Tracheal Stenosis
UES	Upper Esophageal Sphincter

INTRODUCTION

The esophagus is one of the most inscrutable organs of the gastrointestinal tract, hereby it challenges all physicians who specialize on it, including gastroenterologists, surgeons and also primary care physicians. Sometimes patients complain of severe dysphagia or odynophagia without any demonstrable organic background. Another time, overlapping esophageal symptoms, such as heartburn or retrosternal pain, deceive even experienced clinicians and lead to erroneous diagnosis and treatment.

The complexity of the esophagus manifests itself on the colorful spectrum of complications that develop on the ground of gastroesophageal reflux disease (GERD). It is already unexplainable why chronic acid regurgitation provokes reflux esophagitis in some patients, while in others no endoscopic or histologic sign of inflammation can be detected, yet patients have severe complains. Development of diverse functional and structural esophageal disorders have been described in association with gastroesophageal reflux disease, but to date, it is unclear whether these secondary pathologies are simple consequences of GERD or is it possible that some of them are defensive reactions? Is there an expediency in their development or is it mere coincidence? Do the presumed adaptive esophageal changes influence the therapeutic strategy? Gastroesophageal reflux disease has become an endemic malady worldwide in the past decades. Understanding the long-term impacts of acid regurgitation on the esophagus would be imperative in order to better comprehend the behavior of this organ and to improve our knowledge about the possible complications of GERD.

Laparoscopic fundoplication is the standard surgery for gastroesophageal reflux disease. In the short term, fundoplication is considered to be an effective procedure, more than 90% of the patients are satisfied with their symptom control over a period of 1–2 years. However, efficacy wanes with time and post-fundoplication complications may occur, such as migration or herniation of the fundic wrap. These failures aspired us to search for an alternative, possibly more efficient antireflux procedure. With this purpose we set up an innovative surgical antireflux technique, which we carried out on a series of animal models.

Similarly to gastroesophageal reflux disease, lots of questions encircle the development, prognosis and adequate treatment of esophageal cancer. Esophageal cancer is one of the deadliest cancers worldwide due to its extremely aggressive nature and poor prognosis. The incidence of esophageal adenocarcinoma has increased in many western countries, however, esophageal squamous cell carcinoma (ESCC) remains the dominant histological type of esophageal cancer globally. Despite the rapid progress in cancer research, there are still debates concerning the etiology of ESCC. Epidemiological studies suggest that tobacco smoking, heavy alcohol drinking, low socioeconomic status, micronutrient deficiency and dietary carcinogen exposure are the most significant risk factors in the development of the disease. However, the rate of ESCC patients without such history is increasing, which brings up the idea that other environmental factors may be involved in the carcinogenesis. It is also so far unanswered why patients with locally advanced esophageal cancer respond differently to neoadjuvant therapy. Why do upper third esophageal cancers have superior sensitivity to multimodal treatment than lower third cancers? The proximity of the oropharynx and changed sexual habits raise thoughts that human papillomavirus (HPV) infection may play a role in the development of esophageal cancers, similarly as it does in cervical and oropharyngeal cancers. International reports on HPV as a possible etiological factor for esophageal carcinoma are limited and inconsistent, and so is the possible impact of viral infection on the response to the oncological treatment. In order to improve prognosis it would be imperative to find prognostic markers that could distinguish between patients who respond to the oncological treatment and those who don't, consequently saving the non-responder group from unnecessary overtreatment with cytostatics. We searched for answers for these pending problems through retrospective analysis of pre-treatment tumor biopsies of esophageal cancer patients.

During the past quarter century, in the Department of Surgery, University of Pécs, we consulted and operated on nearly two thousand patients with various esophageal disorders. The hypotheses, observations and conclusions of this thesis are based on the experiences gained from the management of these patients.

AIMS

ESOPHAGEAL COMPLICATIONS OF GASTROESOPHAGEAL REFLUX DISEASE: CONSEQUENCES OR DEFENSIVE REACTIONS?

1 We aimed to elucidate our hypothesis whether gastroesophageal reflux disease might induce certain – supposedly adaptive – changes in the esophagus.

2 We aimed to deduce whether the presumed adaptive esophageal changes influence therapeutic strategy.

FUNCTIONAL EXAMINATION OF THE TRANSPOSED PYLORIC SPHINCTER IN ANTIREFLUX PORCINE MODELS: COULD IT BE SUITABLE TO CREATE A CONTINENT ILEOSTOMY?

3 We aimed to ascertain if transposition of a pedicled pyloric sphincter around an impaired gastroesophageal junction is technically feasible and if it is a safe and acceptable antireflux procedure.

4 We aimed to assess if the transposed pyloric sphincter preserves its pharmacological responsiveness in the new, ectopic position.

5 We also aimed to assess a different use of the pedicled pyloric sphincter, namely if it is applicable to complete a 3-limb S-pouch and create a continent ileostomy.

PROGNOSTIC ROLE OF HPV INFECTION IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

6 We aimed to determine the rate of HPV infection in Hungarian patients with locally advanced esophageal squamous cell carcinoma.

7 We aimed to determine the distribution of HPV positivity in the upper, middle and lower thirds of esophageal squamous cell cancer tumor samples.

8 We aimed to compare the response to oncological therapy and also the mean survival of the HPV positive and the HPV negative ESCC patients.

9 We aimed to investigate the correlation between the HPV status and expression pattern of heat shock proteins 90, 27, 16. 2, GHRH-R; and also between expression pattern of heat shock proteins 90, 27, 16. 2, GHRH-R and response to oncological therapy in pre-treatment tumor biopsies of ESCC patients.

CHAPTER 1

ESOPHAGEAL COMPLICATIONS OF GASTROESOPHAGEAL REFLUX DISEASE: CONSEQUENCES OR DEFENSIVE REACTIONS?

1.1 INTRODUCTION

1.1.1 Epidemiology of gastroesophageal reflux disease

Gastroesophageal reflux disease affects approximately 10–20% of the Western population [1], and its prevalence is increasing worldwide due to lifestyle changes, obesity epidemic and population aging. The disease appears to show global variation in its prevalence. For instance, GERD occurs more frequently in the Northern European than in the Southern European population. In Asia the prevalence is reported to be less than 5%, whereas in the United States, more than 40% of the population reports, at least sporadically, symptoms of GERD, hereby GERD being the most common gastrointestinal disorder in the US [2, 3]. Despite the presence of large number of individuals with abnormal gastroesophageal reflux in the general population, primary care physicians, who provide most of their care, continue to have difficulties in recognising the wide range of complications faced by patients with GERD. Besides, this high prevalence has an impact not only on the quality of life, but also on the economy. The burden of GERD in the USA may reach 9–10 billion dollars a year in direct costs, plus uncountable dollars in indirect costs due to decrease in productivity and days off work [4].

1.1.2 Pathophysiology and symptoms of gastroesophageal reflux disease

The pathophysiology of GERD is complex and multifactorial. The disease is on the one hand linked to a misbalance between the protective barriers of the esophageal mucosa and the aggressive chemical substances of gastric refluxate, on the other hand between the valvular mechanism of the gastroesophageal junction and the transdiaphragmatic pressure gradient [5]. The antireflux barriers include two “sphincter” mechanisms: the lower esophageal sphincter and the crural diaphragm that functions as an external sphincter [6].

Malfunction of any of the two may render an individual prone to the development of GERD.

Prolonged exposure of the esophagus to acid and digestive enzymes found in gastric fluid, or duodenal contents regurgitated into the stomach, may induce and promote irritation and result in symptoms and morphological changes to the esophageal mucosa [7]. GERD can manifest in a wide range of symptoms, which can be subdivided into typical, atypical and extraesophageal symptoms. Typical esophageal symptoms include heartburn and acid regurgitation, which have high specificity but low sensitivity for GERD [8]. Atypical esophageal symptoms such as epigastric pain, dyspepsia, nausea, bloating and belching may be suggestive of GERD but may overlap with other conditions in the differential diagnosis such as peptic ulcer disease, achalasia, gastritis, dyspepsia or gastroparesis. Lastly, there are various extraesophageal symptoms including non-cardiac chest pain, chronic cough, asthma, laryngitis and dental erosions [9].

1.1.3 Complications of gastroesophageal reflux disease

Complications of gastroesophageal reflux disease may be esophageal or extraesophageal in nature and may vary from mild esophagitis to major life-threatening problems such as recurrent pulmonary aspiration, Barrett's esophagus or esophageal adenocarcinoma, the latter being one of the most rapidly increasing type of cancer in Western countries [10]. For patients diagnosed with GERD, acid suppression therapy with proton-pump inhibitors is an effective first-line therapy in most cases. However, nearly 40% of the patients have incomplete relief of symptoms even after dose adjustment [11, 12], and in these cases severe complications may develop. The major esophageal complications associated with persistent GERD include erosive esophagitis, ulcerations, strictures, bleeding, Barrett's esophagus and esophageal adenocarcinoma [13]. The extraesophageal manifestations of GERD encompass pulmonary complications, such as asthma [14], ear-nose-throat complications or dental erosions *(Table 1)*.

Table 1. Extraesophageal complications of gastroesophageal reflux [13]

Pulmonary	Cough
	Chronic obstructive pulmonary disease and asthma
Ear, nose and throat	Hoarseness
	Laryngitis
	Sinusitis
Other	Non-cardiac chest pain
	Angina
	Sleep disturbance
	Dental erosion

Thus, chronic acid regurgitation may lead to various secondary pathologies. How does the body react in order to avoid the unwanted, potentially life-threatening complications? To avoid aspiration, the airways are protected by several aero-digestive reflexes such as the esophago-UES contractile reflex, the esophagoglottal closure reflex, and the reflexive pharyngeal swallow [15]. These reflexes provide transient, momentary protection to prevent the potential spillage of refluxate into the airways. Beyond these particular, neuron-mediated quick responses we think that there might be other types of defensive mechanisms. Our hypothesis is that chronic acid exposure may lead to different functional changes in the esophagus (development of hypertensive lower esophageal sphincter, hypertensive upper esophageal sphincter, achalasia or diffuse esophageal spasm) and we propose that these changes could be considered as protective reactions of the organism aimed at guarding the refluxate entering the esophagus or beyond, by narrowing the lumen of the esophagus. A special form of protective reaction can be observed among patients who develop Barrett's esophagus. These patients may experience significant relief of their previous heartburn as Barrett's esophagus develops, and this is due to the replacement of the normal squamous epithelium of the esophagus by acid-resistant metaplastic epithelium [16]. Schatzki's ring, esophageal web and esophageal stricture are also secondary complications of long-standing reflux disease. Although these structural esophageal changes also reduce regurgitation, they can not be considered as adaptive changes, but rather secondary consequences of reflux disease. Cause-and-effect associations between GERD and certain esophageal disorders have already been published in the literature [17, 18, 19].

To our knowledge, this is the first study discussing gastroesophageal reflux disease as a potential causative factor in the development of nine different esophageal disorders that result in reduced acid regurgitation or decreased reflux symptoms. Based on our own experience and review of the literature, we will later on discuss each of these associations individually.

1.1.4 Treatment of GERD

Gastroesophageal reflux disease is a chronic disease that typically requires long term management in the form of lifestyle modification, medical therapy and for a subset of patients, surgical therapy.

Lifestyle and diet modifications traditionally include weight loss, head of bed elevation, avoidance of night-time meals, and elimination of trigger foods such as chocholate, citrus juice, caffeine and alcohol. Although lifestyle changes remain a cornerstone in the initial therapy of GERD, they may not be sufficient to control symptoms in the majority of patients, especially in those with complications.

The mainstay of treatment of GERD is acid suppression, which can be achieved with several classes of medications including antacids, histamine-receptor antagonists (H2RAs) or proton pump inhibitors (PPIs). Studies have shown more complete healing of erosive esophagitis and heartburn relief with PPIs vs. H2RAs and this effect occurs nearly twice as fast (healing rate and heartburn relief of 11.7%/week and 11.5%/week vs. 5.9%/week and 6.4%/week in the PPI and H2RA groups, respectively) [20]. Patients with PPI-refractory GERD can be challenging to treat. In these cases either dosing should be increased or an alternate PPI can be used.

If symptoms persist after attempts at maximizing medical therapy, the evaluation of surgical intervention should be undertaken. Indications for antireflux surgery include unwillingness to remain on lifelong medical therapy, intolerance of medical therapy, medically refractory symptoms with evidence of GERD on endoscopy or pH monitoring, or GERD in the setting of a large hiatal hernia [21]. The two most commonly chosen antireflux surgical procedures are total (Nissen 360°) and partial [anterior (Dor 180°) or posterior (Toupet 270°)] fundoplications. The short and medium term outcomes of laparoscopic antireflux surgery are quite good in terms of improving the typical symptoms of

GERD [22]. However, in the long term it appears these results may diminish. During a mean follow-up period of 9.1 to 10.6 years, a study comparing long term outcomes in medical and surgical therapies for GERD found that 62% of surgical patients took anti-reflux medications on a regular basis, compared to 92% of medical patients [23]. Anti-reflux surgery can be very effective but should not be advised with expectation that patients will no longer take anti-secretory medications [24].

Between 1998 and 2015 a total of 407 patients underwent laparoscopic antireflux surgery at the Department of Surgery, University of Pécs [25]. Complementary hiatoplasty was required in 110 cases (27%). Direct closure of the hiatus was performed in 51 cases, whilst hiatoplasty was reinforced with prosthetic mesh in 28, with teres ligament in 27, and with fascia lata in 4 cases, when direct hiatal closures were not considered reliable. Late postoperative complications necessitated reoperation in 50/407 patients (12%), while 32/110 patients (29%) required revisional surgery due to complications of the hiatoplasty. Probably the most spectacular revisional antireflux surgery was the case of a 68-year-old man who underwent mesh-reinforced antireflux surgery in another institution and presented at our clinic with dysphagia [26]. Examinations revealed partial penetration of the mesh into the esophagus *(Figure 1)*. During an expedited surgery, the mesh was removed through thoracolaparotomy. Distal esophagus and proximal gastric resections were carried out due to longitudinal perforation site and esophageal stricture, and the continuity of the alimentary tract was restored with jejunal interposition.

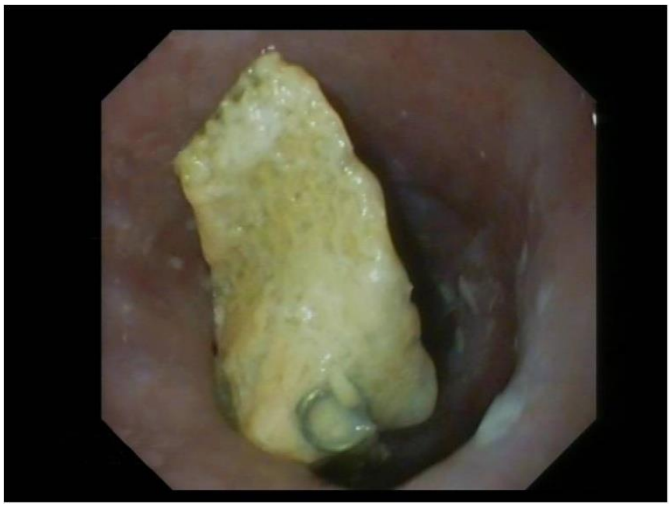

Figure 1. Partial penetration of a sythetic mesh into the esophagus
after mesh-reinforced antireflux surgery /endoscopic image/

To sum up our experiences, we consider that laparoscopic antireflux surgery is a safe procedure with good clinical outcomes. Late complications mostly occur after surgical treatment of large hiatal hernias.

1.2 OBSERVATIONS AND THEORIES

1.2.1 Hypertensive lower esophageal sphincter

To date, probably the most proven protective mechanism of the esophagus against reflux and its complications is the development of a hypertensive lower esophageal sphincter (HLES). In HLES patients LES pressure may increase threefold, as high as 45–60 mmHg. About three-quarters of patients with a hypertensive lower esophageal sphincter have symptoms of regurgitation and heartburn, half have chest pain, and one quarter of patients display an abnormal esophageal acid exposure on 24-hour pH-monitoring [27]. Initially, the association between GERD and HLES may seem paradoxical, as reflux is more commonly associated with a hypotensive, incompetent sphincter. However, HLES is observed in 1.6–2.7% of the patients evaluated for GERD with manometry [17, 28]. These patients often have dysphagia and heartburn simultaneously. The main evidence for HLES being secondary to acid reflux is that following a Nissen fundoplication, sphincter pressure and pH-metry values return to the normal range again and reflux symptoms disappear. Eliminating reflux in HLES patients by performing a fundoplication may at first raise concern about increasing dysphagia, however, the literature justifies its efficiency. Tamhankar *et al.* reported a study determining the outcome of surgical therapy for hypertensive LES associated with GERD or type III hiatal hernia (Group A), and for isolated hypertensive LES (Group B) [29]. Patients in Group A had Nissen fundoplication, and those in Group B had myotomy of the LES with partial fundoplication. At long-term follow-up, dysphagia and chest pain were relieved in all patients. The authors concluded that Nissen fundoplication for hypertensive LES with GERD or type III hiatal hernia relieving dysphagia and chest pain suggests reflux as an etiology in the development of HLES. Katzka *et al.* reported 9 patients with HLES and GERD, in whom LES pressure was restored to normal with anti-reflux medication and an additional three with fundoplication [30].

In our department, between 1999 and 2006 a total of 222 patients underwent laparoscopic fundoplication for GERD, including 6 patients (2.7%) who had manometrically proven HLES [17]. All 6 patients underwent a laparoscopic floppy Nissen fundoplication. Before the operation all 6 patients had abnormal esophageal acid exposure, their mean DeMeester score was 41.7 (range 16.7–86), which returned to the normal mean of 2.9 after the operation. Parallelly, a marked decrease in the gastroesophageal junction pressure was detected. The mean LES pressure before the operation was 50.5 mmHg (range 35.6–81.3), which decreased to a mean of 24.7 mmHg after the surgery. At the late follow-up, all patients were symptom-free.

Thus, some HLES is caused by acid reflux, and this elevated pressure can be interpreted as a protective action of LES to hinder gastroesophageal reflux. By eliminating excessive esophageal acid exposure with a simple fundoplication, the protective reaction of LES is no longer needed, and the pressure of LES returns to normal.

1.2.2 Achalasia

Numerous facts and observations support the theory that there is a cause-and-effect relationship between long-standing reflux disease and the development of achalasia. The theory was first proposed by Smart *et al.* in 1986, who described five patients presenting with reflux and subsequently developing achalasia over the years [31]. Since then several reports were published where gastroesophageal reflux was documented to occur in patients prior to the development of achalasia [32, 33]. Probably the most interesting of these was a case where GERD progressed to diffuse esophageal spasm and then to achalasia [34].

Reflux-induced severe esophagitis may damage the ganglion cells, and later an autoimmune reaction may possibly develop, maintaining chronic inflammation in the myenteric plexus of the esophagus leading to the degeneration of the inhibitory nerve endings and thereby to achalasia. This theory is supported by Altorjay *et al.* who examined morphological and metabolic changes in the muscle samples taken from the LES of reflux patients and compared them to muscle samples taken from non-reflux patients [35, 36]. In the muscle samples of reflux patients, they found a significant increase in the energy-enzyme activities (e.g. creatine kinase, lactate dehydrogenase, beta-hydroxybutyrate dehydrogen-

ase, and aspartate aminotransaminase), as well as in the concentration of structural protein S-100 and the myofibrillar protein troponin; the latter associated with the development of hypertrophy-like changes of the LES muscle. The authors also reported that enteric ganglionitis and significantly lower Schwann cell counts could be detected in LES muscle samples taken from patients with GERD. They suggested that these changes might lead to various functional esophageal diseases.

Other reports showed that 10–20% of untreated achalasia patients had abnormal acid exposure on pH monitoring [36, 37, 38]. These acidic episodes were accompanied by transient relaxations of the LES [39]. In the literature the development of Barrett's esophagus [40] and even esophageal adenocarcinoma among untreated achalasia patients has been reported, suggesting that long-standing reflux disease preceded, and possibly played an etiological role in the development of achalasia. Similarly, several cases have been described where achalasia occurred with concomitant hiatal hernia [41, 42]. It is well-known that hiatal hernia facilitates development of GERD due to impairment of antireflux barriers, namely the elongation of the phrenoesophageal ligament and migration of the gastroesophageal junction from the abdominal cavity into the thorax, the coexistence of hiatal hernia and achalasia seeming to be paradoxical. Despite the apparent contradiction, we also had patients with achalasia and concomitant hiatal hernia *(Figure 2)*.

In summary, the potential relationship between gastroesophageal reflux and achalasia is not clearly defined yet, but we have reason to believe that it can very well be. Based on a rational interpretation of our own findings and literature *(Table 2)*, it can be assumed that chronic acid exposure may trigger diverse structural and functional changes in the esophagus.

Our surgical team has operated on over 40 patients with achalasia in the past 15 years, and in 10% of the cases achalasia developed on the ground of gastroesophageal reflux disease. In our opinion, in these cases dilation therapy should be avoided and the surgical management of achalasia should include a laparoscopic Heller's myotomy completed with a 360° Nissen fundoplication in order to minimize the risk of postoperative reflux.

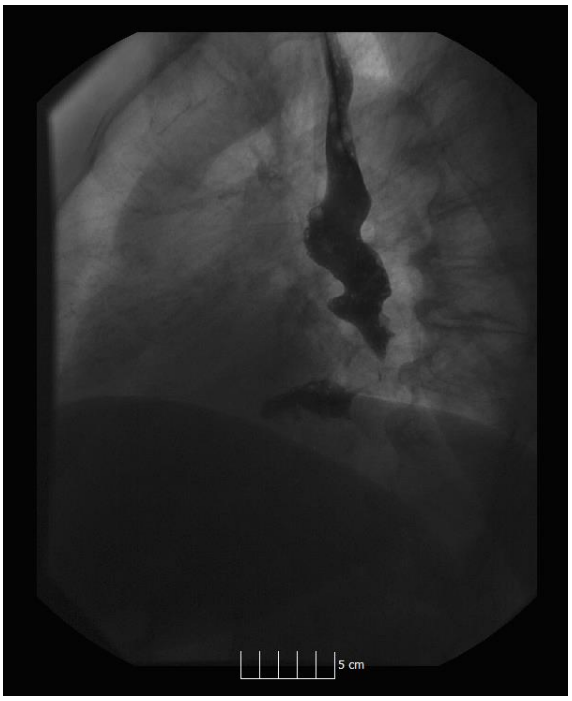

Figure 2. Achalasia and concomitant hiatal hernia on barium swallow test

Table 2. Literature analysis of the connection between gastroesophageal reflux disease and achalasia

Study	Number of patients	Conclusion
Smart et al,[30] 1986	5	Causative connection
Altorjay et al,[34] 2006	11	Similar morphologic changes
Robson et al,[33] 2000	1	Causative connection
Schoenut et al,[31] 1995	23	Possible connection
Guo et al,[39] 2002	2	Causative connection
Varga et al,[17] 2008	6	Causative connection
Lamb et al,[27] 2009	30	No relation presumed
Moses et al,[43] 2003	45-16 [a]	No connection
Fischiella et al,[44] 2008	7	No relation presumed
Spechler et al,[45] 1995	9	Coincidence

Note: [a] In this paper 45 cases with achalasia and 16 cases with GERD were reported.

1.2.3 Diffuse esophageal spasm

Diffuse or distal esophageal spasm (DES) is an uncommon esophageal motility disorder that presents clinically with chest pain and/or dysphagia and is defined manometrically as simultaneous contractions of the distal esophagus in ≥20% of wet swallows alternating with normal peristalsis [46]. DES was described clinically more than 100 years ago by Osgood *et al.* [47] and manometrically in 1954 by Creamer *et al.* [48]. Despite the recognition of the condition for over a century, the cause of the disease remains unknown. Some studies have suggested that DES is characterized by a loss of neural inhibition [49]. Functional studies in animal and human models have found that inhibition of nitric oxide induces simultaneous contractions in the distal esophagus, the manometric hallmark of DES, whereas replacement of nitric oxide reverses the defect.

The role of gastroesophageal reflux disease in DES, has been suggested by several observations. Esophageal motility abnormalities induced by acid perfusion have been described since the late '60s [50]. As we mentioned before, a very special and spectacular case was reported by Robson *et al.* who presented a patient with gastroesophageal reflux disease, who subsequently developed diffuse esophageal spasm and then achalasia [34]. Coexisting gastroesophageal reflux disease has been reported to occur in 38–60% of patients with DES [51, 52]. Treatment of DES is imperfect and difficult due to the incomplete understanding of the pathophysiology and cause of this condition. Although there is no consensus on the role of acid reflux in DES, patients with coexisting GERD are suggested to use acid suppression instead of muscle relaxants, which may worsen their GERD. We believe that in case of patients where the above characterized simultaneous esophageal contractions develop on the ground of gastroesophageal reflux disease, DES can be considered as an adaptive reaction of the esophagus, as the contractions will after all impede the gastric refluxate to get into the esophagus or above.

We had a 70-year-old male patient presenting with epigastric pain and progressive dysphagia. Examinations revealed diffuse esophageal spasm with hiatal hernia and GERD *(Figure 3)*.

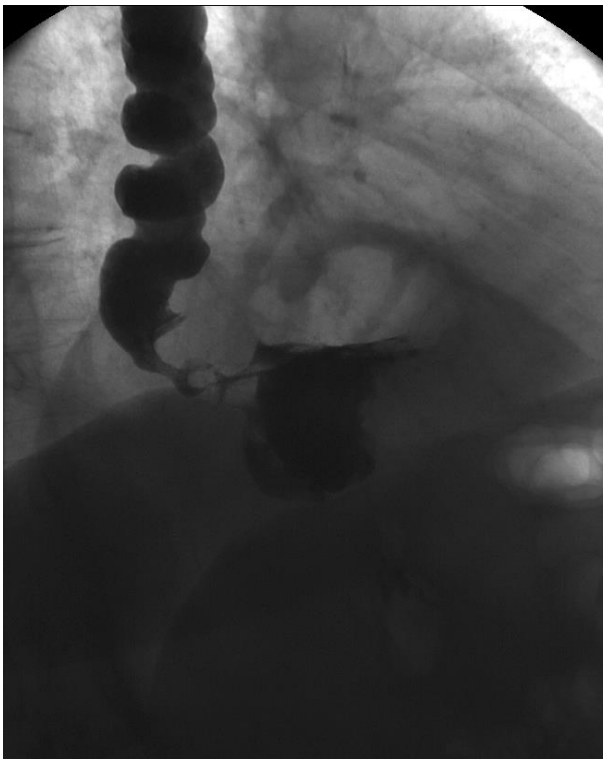

Figure 3. Diffuse esophageal spasm and concomitant hiatal hernia on barium swallow test

1.2.4 Hypertensive upper esophageal sphincter

Several studies have reported a swift rise in upper esophageal sphincter (UES) pressure following the infusion of acid into the lower esophagus of healthy subjects [53, 54]. Tokashiki *et al.* [55] termed this reflex-based rise in UES pressure "respiratory defense mechanism" in which the presence of acid is first detected by the mucosal afferent nerves of the UES, then the sphincter contracts reflexively to protect the respiratory tract from aspiration. We presume that chronic acid exposure in the esophageal body likely leads to the hypertonicity of the UES, which can be considered as an adaptive mechanism.

The leading symptoms of patients with a hypertensive UES (HUES) are cervical dysphagia and nocturnal cough. If the patient gives an account of long-standing heartburn prior to these complaints, the likelihood of a causal relationship between chronic GERD and HUES is increased. In case of reflux induced HUES treatment strategy should in all cases comprise long-term acid suppression therapy after the esophageal dilation.

We had two patients with such history. In one patient examination revealed a 3 cm long narrowing of the esophageal lumen at the pharyngoesophageal junction *(Figure 4)* and concomitant gastroesophageal reflux disease, shown by 24h pH-metry. The assump-

tion that reflux induced the formation of the hypertensive UES was confirmed by the fact that the patient became symptom-free following a dilation and long-term PPI therapy. The other patient also presented with complaints of escalating cervical dysphagia. Barium swallow test revealed a cricopharyngeal bar *(Figure 5)*, which is the hypertrophy of the cricopharyngeus muscle, while 24h pH-metry showed abnormal reflux patterns. Similarly to the previous case, this patient also underwent esophageal dilation, was put on acid suppression therapy and from then on he became symptom-free.

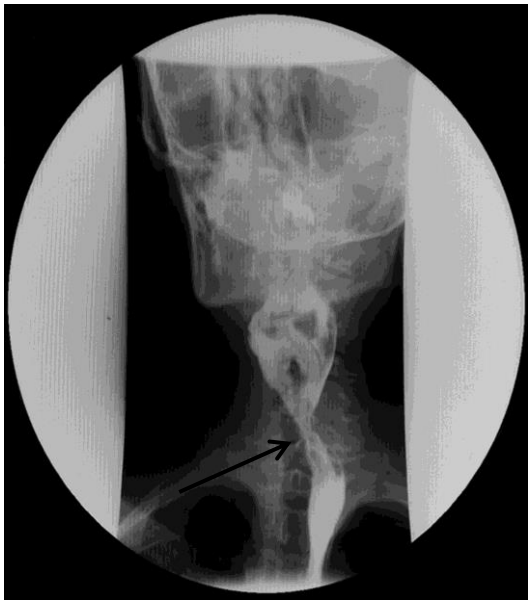

Figure 4. Narrowing of the upper esophageal sphincter due to hypertonicity of the UES /barium esophagogram/

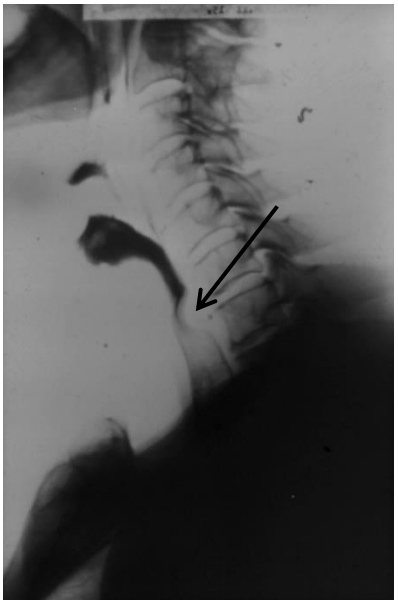

Figure 5. Cricopharyngeal bar shown on barium swallow test

1.2.5 Zenker's diverticulum

A large body of evidence supports the association between GERD and the development of a Zenker's diverticulum, a pharyngeal pouch formed from a weak point in Killian's triangle [19]. The formation of this diverticulum of the hypopharynx is the consequence of cricopharyngeal muscle hypertension, which is most likely induced by chronic acid exposure and can be regarded as a defensive mechanism of the esophagus precluding aspiration. The development of a Zenker's diverticulum is an unwanted, indirect conse-

quence of this protective hypertension of the cricopharyngeal muscle. Morales-Divo *et al.* found extraesophageal reflux in more than 72% of the patients with Zenker's diverticulum [56]. The same author reported that hiatal hernia was found in 39% of the patients with Zenker's diverticulum, while it occured only in 16% in the control group. Approximately 10% of patients undergoing antireflux surgery have short esophagus [57]. Sasaki *et al.* presumed that acid reflux may induce longitudinal esophageal shortening, which in turn increases the risk for the development of herniation between two spatially related structures, the pharyngeal constrictors and cricopharyngeus muscles, leading to the development of Zenker diverticulum [19].

We recommend that when establishing the diagnosis of a Zenker's diverticulum, the presence of GERD should be always excluded. If reflux is present, therapy should comprise either endoscopic or surgical treatment of the diverticulum, completed with cricopharyngeal myotomy and long-term acid suppression therapy.

A long peptic stricture and a Zenker's diverticulum *(Figure 6)* were revealed to be the cause of progressive dysphagia and regurgitation symptoms of a male patient who presented at our clinic. Both disorders were considered to be consequences of GERD.

Figure 6. Long peptic stricture and a Zenker's diverticulum shown on barium swallow

1.2.6 Schatzki's ring

Schatzki's rings are lower esophageal rings that form at the esophagogastric junction and are thin concentric protrusions covered proximally by normal esophageal squamous epithelium and by gastric epithelium on the distal side of the membrane [58]. It is well established that 31–66% of SR patients are found to have pathologic gastroesophageal reflux on 24-h esophageal pH monitoring [18, 59] and nearly all of them have hiatal hernia [60]. The current, established treatment for Schatzki's rings is esophageal dilation. Although this therapy is effective initially, long-term results are disappointing with high symptomatic relapse rates [61]. Several studies and anecdotal evidence suggested that dilation or incision in patients with symptomatic lower esophageal rings resulted in longer symptom-free survival and fewer recurrences if those were followed by treatment of GERD [60, 62]. This finding was confirmed by Sgouros *et al.* in a prospective study [63]. They found that the high relapse rate after successful esophageal dilation due to the reformation of the ring could be prevented by a post-dilational, long-term, acid-suppressive maintenance therapy with PPIs. These findings support an inflammatory etiology of the ring, promoted by acid reflux in the esophagus. We do not consider that the development of Schatzki's ring in reflux patients is an adaptive reaction, however, it clearly reduces acid exposure into the upper parts of the esophagus.

An interesting, but unsurprising observation was made by Mitre *et al.*, namely that Barrett's esophagus is less prevalent in patients with Schatzki's ring compared to patients without Schatzki's ring (0.73% vs. 1.80%) [64]. Long segment Barrett's esophagus was not observed in any patients with Schatzki's ring. This observation supports the theory that Schatzki's ring protects the esophagus proximal to the ring from excessive acid exposure.

In our clinic we treated eleven patients with Schatzki's ring. All of them had symptoms of GERD prior to the development of dysphagia, and at diagnosis barium swallow test revealed a concomitant hiatal hernia in nearly all cases *(Figure 7)*.

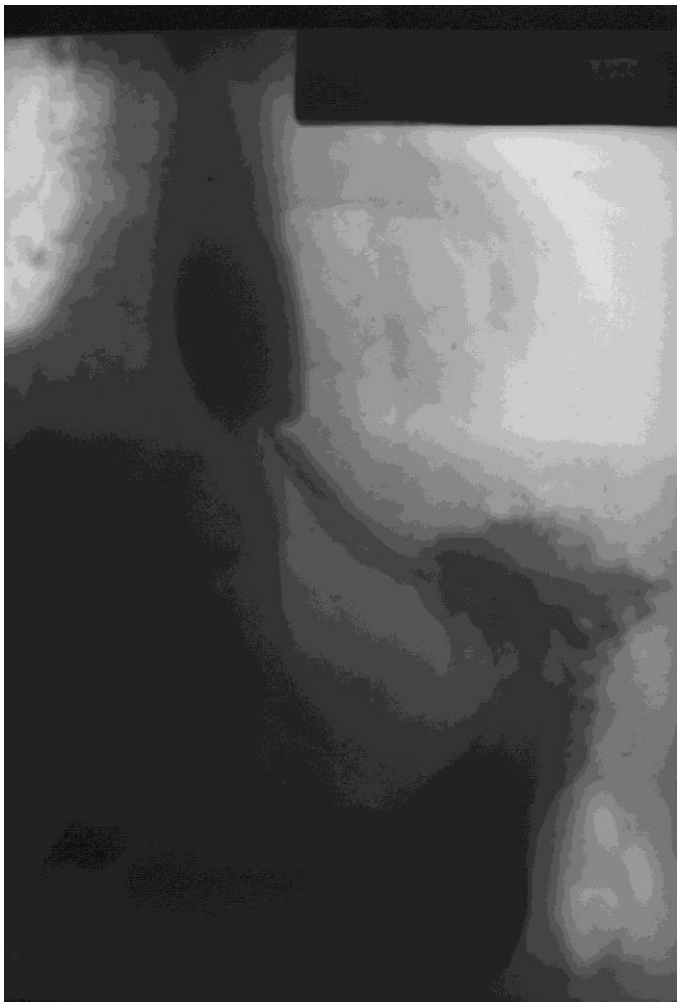

Figure 7. Typical Schatzki's ring and concomitant hiatal hernia /barium esophagogram/

1.2.7 Esophageal web

An esophageal web is a thin, eccentric, smooth extension of normal esophageal tissue consisting of mucosa and submucosa that can be found anywhere along the esophagus, but typically occur in the proximal esophagus. Similarly to Schatzki's ring, esophageal webs could be considered as secondary consequences of GERD, which ease the patient's sypmtoms of heartburn, however, their formation can not be regarded as a real adaptive mechanism.

We managed a 42-year-old male patient at our clinic who presented with progressive dysphagia. An esophageal web was diagnosed *(Figure 8)*. We excluded the presence of Plummer-Vinson syndrome, NSAID or caustic injury in the patient's history. The web was successfully treated with dilation. The subsequent onset of reflux symptoms suggested reflux as an etiology, which ceased as soon as the patient was put on omeprazol therapy. Reformation of the web was not observed.

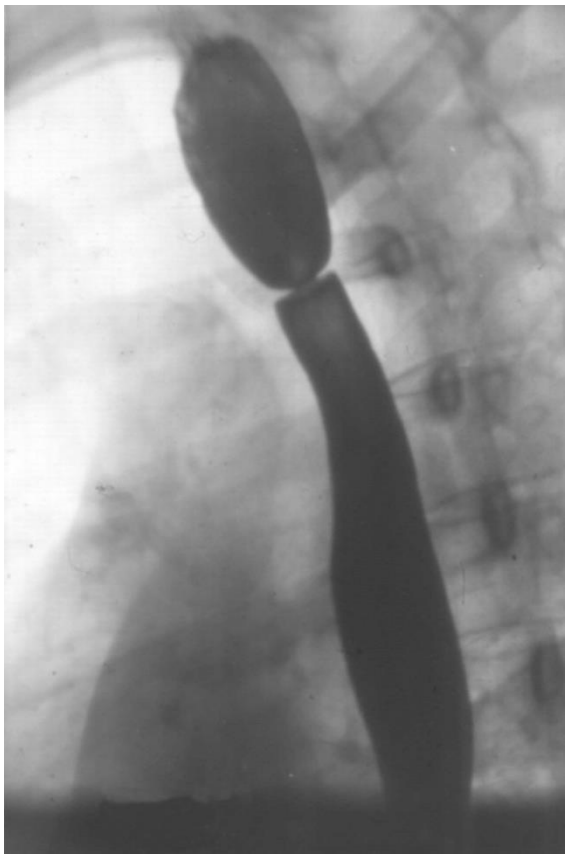

Figure 8. Esophageal web between the upper and middle third of the esophagus /barium swallow/

1.2.8 Barrett's esophagus

The development of Barrett's esophagus can be regarded as a special form of accommodation of the esophagus against reflux. The metaplastic specialized columnar epithelium (SCE) of Barrett's esophagus arises in the setting of long-lasting GERD likely as replacement for acid-damaged squamous epithelium (SqE). As such, SCE is more acid-resistant than SqE and represents a form of "adaptive protection" against a hostile luminal environment [16]. Consequently the long-lasting heartburn and other reflux symptoms may ease as soon as patients develop Barrett's esophagus due to the insensitivity of the specialized columnar epithelium. This has been clinically demonstrated by use of the Bernstein test [65]. This observation is also supported by routine histological findings, namely that biopsies taken from the intestinalized cardiac mucosa show little or no inflammation. DeMeester presumed that the widespread use of acid-suppressive medication since 1970s, played a role in the increasing incidence of Barrett's esophagus [66]. According to this theory, elevation of the pH up to the 3–6 range stimulates phenotypic differentiation of the cardia type mucosa toward intestinalization [67]. This more alkaline gastric environment causes the biochemical imbalance of bile acid metabolism, which may result in an increased probability of mucosal damage. Also, this injury may lead to genetic modifications in the progenitor cells, which may subsequently produce metaplastic epithelium. Jovov *et al.* examined what contributed to the relative acid resistance of SCE in Barrett's esophagus compared with the native SqE vulnerable to acid damage. They found that the SCE of Barrett's esophagus is significantly richer in Claudin-18 (a dominant tight junction protein) than SqE and this may contribute to its greater acid resistance, as Claudin-18 selectively decreases the permeation of cations through the paracellular pathway and this decrease extends to H^+ [16]. Nancarrow *et al.* studied the genome expression profile of esophageal biopsy tissues from individuals with Barrett's esophagus and those with normal squamous epithelium [68]. They found that Barrett's esophagus is a tissue with capacity for enhanced glycoprotein synthesis machinery designed to provide strong mucosal defenses aimed at resisting gastroesophageal reflux disease.

1.2.9 Esophageal stricture

Esophageal strictures are well-known severe complications of GERD that occur in approximately 10% of patients with untreated erosive esophagitis [69]. Strictures have been associated with hypotensive lower esophageal sphincter, hiatal hernia, motility dysfunction and bile reflux exposure [70]. They usually occur in the lower esophagus and typically tend to be 2–4 cm long, but may include the entire length of the thoracic esophagus [71]. The pathogenesis of esophageal strictures is unclear. Most theories say that peptic strictures develop as a result of healing and fibrosis of reflux induced inflammatory lesions in the distal esophagus, leading to esophageal wall thickening and luminal constriction. Most strictures cease to worsen when the esophagus is narrowed to a diameter of 1.5–2 cm [71]. This near-complete lumen obliteration limits the gastric refluxate to go up to the proximal esophagus and beyond.

We observed in several of our own patients that as soon as the peptic stricture develops, esophagitis being proximal to the narrowing ceases *(Figure 9)*. We believe that in case of reflux induced esophageal strictures, the luminal constriction is not an adaptive reaction against reflux, but a structural consequence of acid induced inflammation that will, as a matter of fact, finally lead to a reduced risk of pulmonary aspiration.

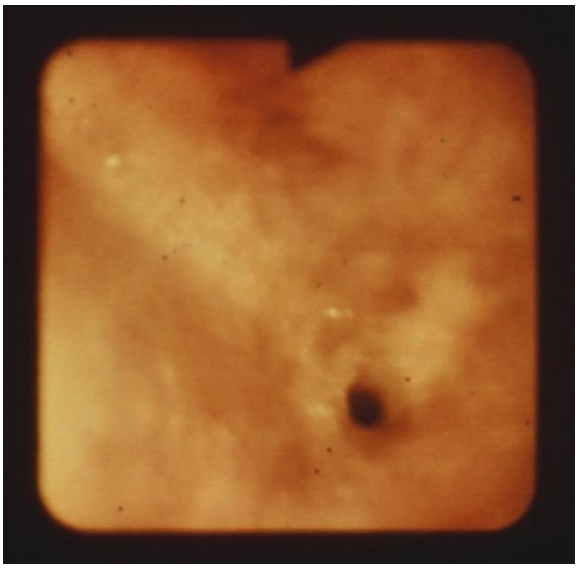

Figure 9. Esophagitis ceased proximal to the esophageal stricture /endoscopic image/

1.2.10 Subglottic tracheal stenosis

Several studies reported a strong association between extraesophageal reflux (EER) and development of subglottic tracheal stenosis (STS) [72-76]. Subglottic tracheal stenosis is not an esophageal pathology, therefore it can be considered as an odd one out in this thesis, however, narrowing of the upper airway in response to acid reflux can also be considered as a form of protective reaction against aspiration, as such we find it important to mention here. Subglottic tracheal stenosis is an abnormal narrowing of the airway in between the glottis and the cricoid cartilage. It may be due to a variety of diseases, such as post intubation injury, airway trauma, inhalation burns or irradiation. A peculiar group of patients with subglottic stenosis is represented by those with "idiopathic" stenosis, who, by definition have no known etiology. This disease is characterized by an inflammatory cicatricial stenosis that occurs almost exclusively in females between the third and fifth decade [77]. Extraesophageal reflux has been implicated as a contributing or causative factor in the development of inflammatory lesions of the upper aerodigestive tract over the last several decades. In 1980, Bogdasarian *et al.* suggested that gastroesophageal reflux is a factor significant to the development of posterior glottic stenosis [72]. Koufman *et al.* reported a study involving 32 patients with laryngotracheal stenosis (LTS) and found that 78% of the patients demonstrated laryngopharyngeal reflux with dual-probe 24-hour pH-metry [73]. In a prospective study, Toohill *et al.* concluded that reflux to the pharynx, larynx and trachea would add insult to an intubated or injured airway, thus enhancing the development of a stenosis [74]. The authors found that identification and treatment of GER simplifies and improves the treatment of LTS and they suggested that all patients with LTS should be concomitantly managed for GERD. Jindal *et al.* studied seven patients with idiopathic subglottic stenosis (iSGS) by barium esophagogram, pH monitoring, and response to antireflux therapy and concluded that GER was the likely cause of iSGS [75]. Strong evidence of the presence of EER in iSGS was published by Blumin *et al*. [76]. In a prospective study involving 22 patients with iSGS, biopsies of the subglottic scar and postcricoid area were evaluated for the presence of pepsin, which is well-known to be made only by gastric mucosa. They found that one-half of the patients had pepsin embedded in the tissue of their larynx or subglottic scar. This finding was substantial and

gave support that this condition is indeed a result of refluxed and microaspirated gastric contents.

Luminal stenosis of the subglottic trachea in response to EER decreases the risk of aspiration in the lower airways. In our interpretation, subglottic tracheal stenosis can be classified as a reflux induced structural consequence, which by the way, reduces the risk of aspiration.

We had an interesting case of a 70-year-old woman who used to have achalasia and for this reason underwent esophagogastrostomy 40 years ago. Thereafter she developed severe acid reflux with pulmonary complications and a subglottic trachea stenosis that even necessitated tracheostomy provisionally. Regular intake of antacid drugs led to alleviation of the stenosis, subsequently the tracheostomy was closed. Forty years later she presented at our hospital with stridor and difficulty breathing and a subglottic tracheal stenosis was revealed again *(Figure 10)*. We presumed that reformation of the stenosis was triggered by recurrent reflux originated from the esophagogastrostomy. Accordingly we resected the anastomosis and restored the continuity with a jejunal interposition. The patient's reflux symptoms ceased and the tracheal stenosis was treated by dilation.

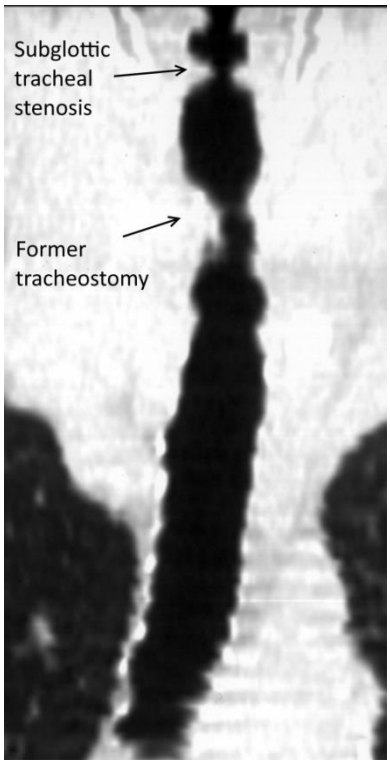

Figure 10. Subglottic trachea stenosis and below
the former site of tracheostomy /virtual bronchoscopy/

1.3 CONCLUSION

In summary, the esophagus – and the airway – can react in various ways to chronic acid exposure [78-81]. Patients with progressive GERD, refractory to medical treatment, may develop diverse structural and functional esophageal changes *(Table 3)*. The functional changes (HLES, HUES, achalasia, diffuse esophageal spasm) seem to be adaptive reactions aimed at easing the unpleasant symptoms and reducing acid regurgitation. The development of Barrett's esophagus can also be regarded as an adaptive change, as it is associated with a decrease in reflux symptoms. The structural changes (Schatzki's ring, esophageal web, esophageal stricture, subglottic tracheal stenosis) also result in decreased acid regurgitation, but we consider that these are rather secondary consequences of GERD and not adaptive mechanisms. We have to note that these apparently protective complications may also give rise to some inconvenient conditions. For instance, Barrett's esophagus is the anteroom of esophageal adenocarcinoma. Or, the newly developed esophageal motility disorders are often followed by dysphagia and chest pain, or, the hypertonicity of the upper esophageal sphincter might facilitate the development of a Zenker's diverticulum, leading to further complications.

We recommend that when establishing the diagnosis of a functional esophageal disorder, the etiology of GERD should be at all times ruled out, as it may alter therapeutic strategy. If reflux proves to be present, treatment should primarily focus on eliminating acid regurgitation.

In human evolution, we observe countless forms of adaptation. However, the development of adaptive or defensive reactions in ontogeny is very much debated in the literature. At the same time, the enumerated protective reactions suggest that these changes are probable accommodation mechanisms of the organism, aimed at preventing the harmful consequences of reflux disease. It is unlikely that these are simple coincidences. However, we have no satisfactory explanation as to that why some reflux patients will develop HLES, while others develop achalasia or HUES, etc. in response to acid reflux. Similarly, no explanation can be provided for why only half of reflux patients develop esophagitis and the other half do not. Could the difference be found in the composition of the refluxed juice or in the patients? This is a highly interesting research question. We believe that the hypothesis that GERD may induce self-protective reactions in the esophagus is

well-supported by the findings detailed in this thesis, but further large-scale prospective studies are needed to confirm these associations.

Table 3. GERD induced protective reactions

Adaptive changes	Structural changes
Hyperensive lower esophageal sphincter	Schatzki's ring
Achalasia	Esophageal web
Hypertensive upper esophageal sphincter - Zenker's diverticulum	Esophageal stricture
Barrett's esophagus	Subglottic tracheal stenosis
Diffuse esophageal spasm	

CHAPTER 2

FUNCTIONAL EXAMINATION OF THE TRANSPOSED PYLORIC SPHINCTER IN ANTIREFLUX PORCINE MODELS: COULD IT BE SUITABLE TO CREATE A CONTINENT ILEOSTOMY?

2.1 INTRODUCTION

The pyloric sphincter is a cone-shaped band of circular smooth muscle at the gastroduodenal junction that acts as a valve, permitting food to pass into the duodenum or be retained in the stomach. When removed from the gastroduodenal continuity, the pyloric valve looses its parasympathetic innervation, which results in an increased resting tone of the ring. After careful consideration of the benefits and disadvantages, the pyloric valve can be sacrificed in order to, for example, replace another damaged sphincter in the body. The successful transposition of the pyloric sphincter to reconstruct or replace a severely damaged incontinent anal sphincter has been previously described in humans and it was suggested as a surgical option for patients with end stage fecal incontinence [1, 2]. Removal of the sphincter from its original place does not significantly influence the quality of life, distal partial gastrectomy is described to have a tolerable amount of late complications. To date, distal gastrectomy with Billroth I reconstruction is a generally accepted surgical approach for both early distal gastric cancer and complicated peptic ulcer disease [3]. Billroth I type of anastomosis is reported to be associated with lower rate of early postoperative complications compared to Billroth II and Roux-en-Y reconstructions [4]. Furthermore, pyloric valve transposition leaves the antrum intact, therefore it is a less radical intervention than Billroth I surgery.

In our study, we planned a two-stage animal experiment aimed at examining different uses of the pyloric sphincter. The primary purpose of our experiment was to evaluate whether the pyloric sphincter based on the left gastroepiploic arterial pedicle could be technically transposed around the esophagogastric junction that had been previously weakened by circular myectomy, hereby artificial gastroesophageal reflux was created. Today laparoscopic fundoplication is the standard surgery for gastroesophageal reflux

disease. In the short term, fundoplication is considered to be an effective procedure, more than 90% of the patients are satisfied with their symptom control over a period of 1–2 years [5]. However, efficacy wanes with time and post-fundoplication complications may occur, such as migration or herniation of the fundic wrap. These failures aspired us to search for a different, possibly more efficient antireflux procedure. We investigated whether creation of a pyloric wrap could be an alternative of fundoplication. Beyond the technical feasibility, we examined if the valve preserved its pharmacological responsiveness in the new, ectopic position.

In the second series of our experiment we aimed to examine the use of the pyloric ring in the construction of a continent ileostomy, where there would also be great need for a functioning sphincter to achieve complete continency. Continent ileostomy is an alternative to end ileostomy for patients who have undergone total proctocolectomy, providing substantial physical and psychosocial benefits over a conventional ileostomy. The surgical technique to create a continent ileal reservoir has evolved in the past decades, however, complete continency can still not be achieved, and regular pouch intubation is needed [6], leaving millions of people with reduced quality of life worldwide. Wrapping a pedicled pyloric sphincter around the efferent loop of a small intestine reservoir may probably guarantee constant continency, whilst local use of smooth muscle relaxant could allow planned evacuation of the stoma.

2.2 METHODS

2.2.1 Antireflux model

In the first series of our experiment 6 pigs (Hungahib-39 pigs, BW: 20.1 +/- 1.9 kg) underwent surgical transposition of a pedicled pyloric sphincter around the gastroesophageal junction, where circular myectomy, hereby artificial gastroesophageal reflux had been previously created. The experiment was performed in the Surgical Research Laboratory of the University of Debrecen, Hungary. The operations were carried out under general anesthaesia. As premedication a single dose of *azaperon* (2 mg/kg) was given intramuscular. The anesthesia was maintained by giving intramuscular *ketamine* (15 mg/kg) and *xylazine* (1 mg/kg) throughout the experiment. In all experimental animals an inferior

tracheostomy was performed and an endotracheal tube was inserted for supported ventilation. Pressure support mechanical ventilation (Airox Legendair Ventilator, PAU Cedex France) was used. After a midline laparotomy, the gastrocolic omentum was dissected off the transverse colon. The branches of the gastroepiploic vascular arcade to the greater curvature of the stomach were divided with care taken to preserve the epiploic arcade and terminal branches to the pyloric valve. Mobilization of the gastroepiploic arcade started at the upper margin of the pylorus, detachment then proceeded to include one-half to two-thirds of the greater curvature. The right gastroepiploic artery was ligated and divided at the lower margin of the pylorus. The antral and duodenal ends of the pyloric valve were cut *(Figure 11)*. The pyloric ring was, from here on, based on the left gastroepiploic artery. Gastroduodenostomy was performed to restore the gastrointestinal continuity.

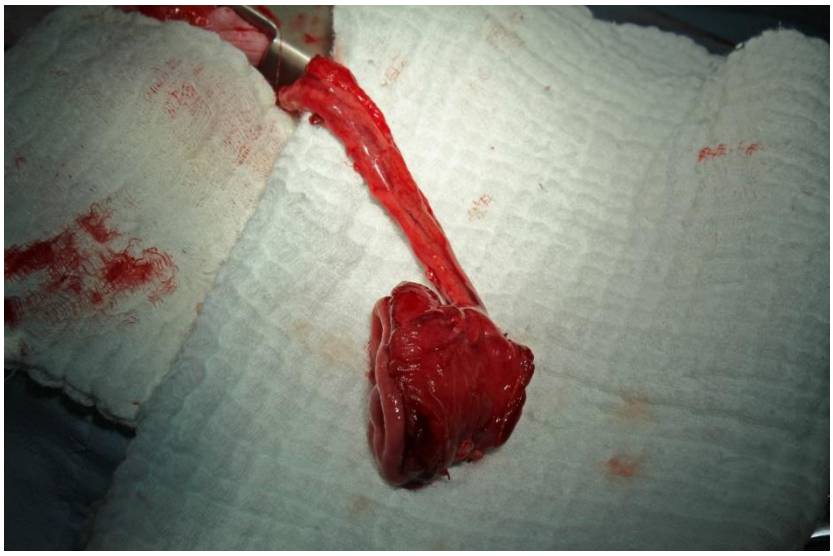

Figure 11. Pyloric ring based on the left gastroepiploic artery

After this, we excised the muscular layer of the lower esophageal sphincter on a zone as wide as 1.5 cm. The pyloric ring was cut on the lesser curvature side, and sutured around the impaired distal esophagus *(Figure 12)*. In all animals, changes in the microcirculation of the pyloric ring were measured by laser Doppler flowmetry (LDF) before and after the transposition. Similarly, changes in the intraesophageal pressures were measured before and after the cardiomyectomy and after the transposition of the ring around the cardia. For this, we used a multichannel esophageal balloon manometry, custom-made and developed by the Department of Information Technology, University of Debrecen,

Hungary. After this, half of the animals received parasympathomimetics (*neostigmine* 0.2 mg), the other half nitrovasodilators (*glyceryl trinitrate* 4 mg) systematically, then the intraesophageal pressure and microcirculation of the transposed pylorus were measured again.

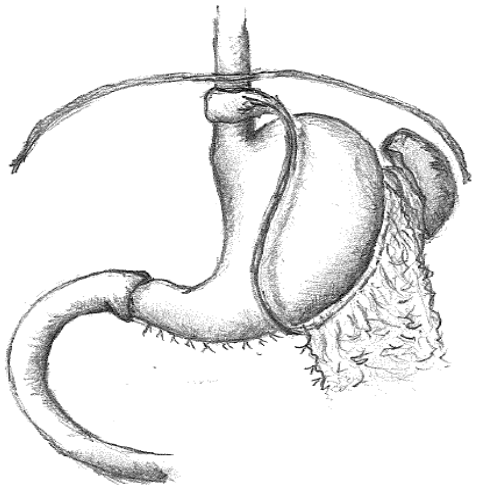

Figure 12. Pyloric sphincter based on the left gastroepiploic artery and transposed around the weakened gastroesophageal junction

The experiment was approved and registered by the University of Debrecen Committee of Animal Welfare (permission Nr.: 22/2017. UD CAW), in accordance with the Hungarian Animal Protection Act Law XVIII/1998 and the Ordinance 40/2013. (II.14.) of the Hungarian Government and EU directive (Directive 2010/63/EU).

2.2.2 Continent ileostomy model

In the second series of our experiment, as a preliminary study, we aimed to create continent ileostomy in porcine models with the use of a pedicled pyloric sphincter, wrapped around the efferent loop of a 3-limb S-pouch. We involved 2 female pigs in the study who were under the same general anesthesia as in the first series of the experiment. After a midline laparotomy, an end ileostomy with an S-pouch was created in both subjects. The S-pouch included three 10-cm small bowel limbs to construct the reservoir, and the efferent limb of 15 cm was sutured to the abdominal wall as a stoma. The pyloric ring based on the left gastroepiploic arterial pedicle was dissected the same way as described before,

the pylorus was then cut on the anti-mesenteric side and placed next to the ileostomy, where it was wrapped around the small intestine between the abdominal wall and the S-pouch *(Figure 13)*. The diameter of the pyloric ring was adjusted to the diameter of the ileum in a way that a 24 Ch catheter was inserted in the lumen of the ileostomy and the pylorus was wrapped tightly around the bowel, with regard to avoid ischaemization of the intestine. The catheter was left behind in the intestine in order to relieve the anastomosis. Pharmacological responsiveness of the transposed pyloric ring to drugs was not examined in these cases.

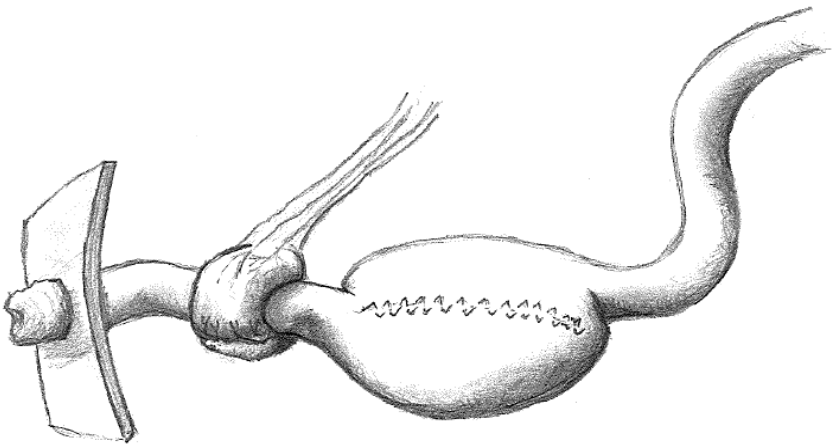

Figure 13. Pyloric sphincter based on the left gastroepiploic artery and transposed around the efferent loop of a S-pouch

2.3 RESULTS

Transposition of the pedicled pyloric ring around the impaired cardia is technically feasible, the pyloric flap preserved its viability throughout the operation *(Figure 14)*. However, the lumen of the ring was too narrow to surround appropriately the esophagus, and most likely would have caused dysphagia on the long term. The pharmacological responsiveness of the ring in the ectopic position, however, could be examined very well.

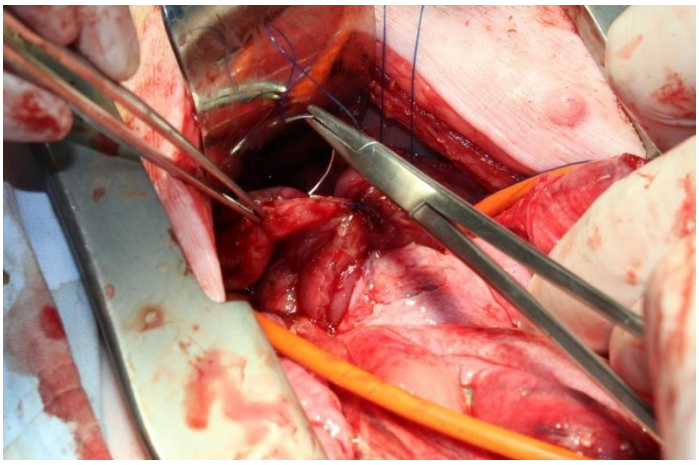

Figure 14. Pyloric sphincter being sutured around the esophagogastric junction

Microcirculation of the sphincter with the gastroepiploic artery (GEA) pedicle barely changed after it was removed from the gastroduodenal continuity (33 BFU vs. 32 BFU), which proves the completeness of the gastroepiploic arcade *(Figure 15)*. After the creation of the pyloric cuff around the weakened cardia, 3 animals were given *glyceryl trinitrate* 4 mg (Group A), while the other 3 received *neostigmine* 0.2 mg intravenously (Group B). 3 minutes later, laser Doppler flowmetry showed that microcirculation of the ring increased in Group A (40 BFU) and decreased in Group B (28 BFU). Parallelly, we measured the intraesophageal pressure at different stages of the operation *(Figure 16)*. The average basic pressure of the intact cardia was 42 mmHg, which dropped to an average of 26 mmHg after the partial cardiomyectomy. Then, as the cut pyloric ring was sutured around the weakened cardia with its original diameter, the intraesophageal pressure rose to an average of 72 mmHg. This value suggests that the ring is too narrow to use as a cuff around the esophagus, and would probably cause dysphagia in the animal. In Group A, 3 minutes after the injection of *glyceryl trinitrate*, the intraesophageal pressure dropped to an average of 65 mmHg. In Group B, the injection of *neostigmin* led to further increase in the intraesophageal pressure after 3 minutes (84 mmHg).

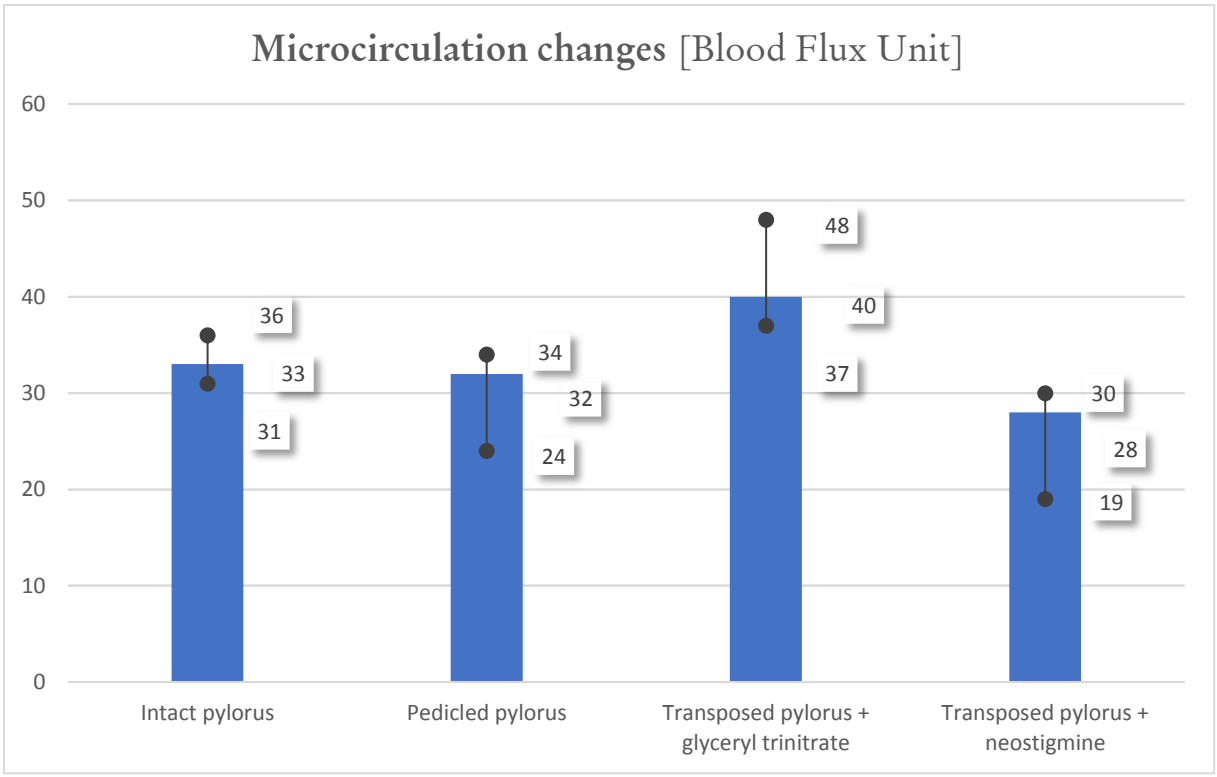

Figure 15. Changes in the microcirculation of the pyloric ring measured by Laser Doppler (minimum, mean and maximum BFU values in *au*)

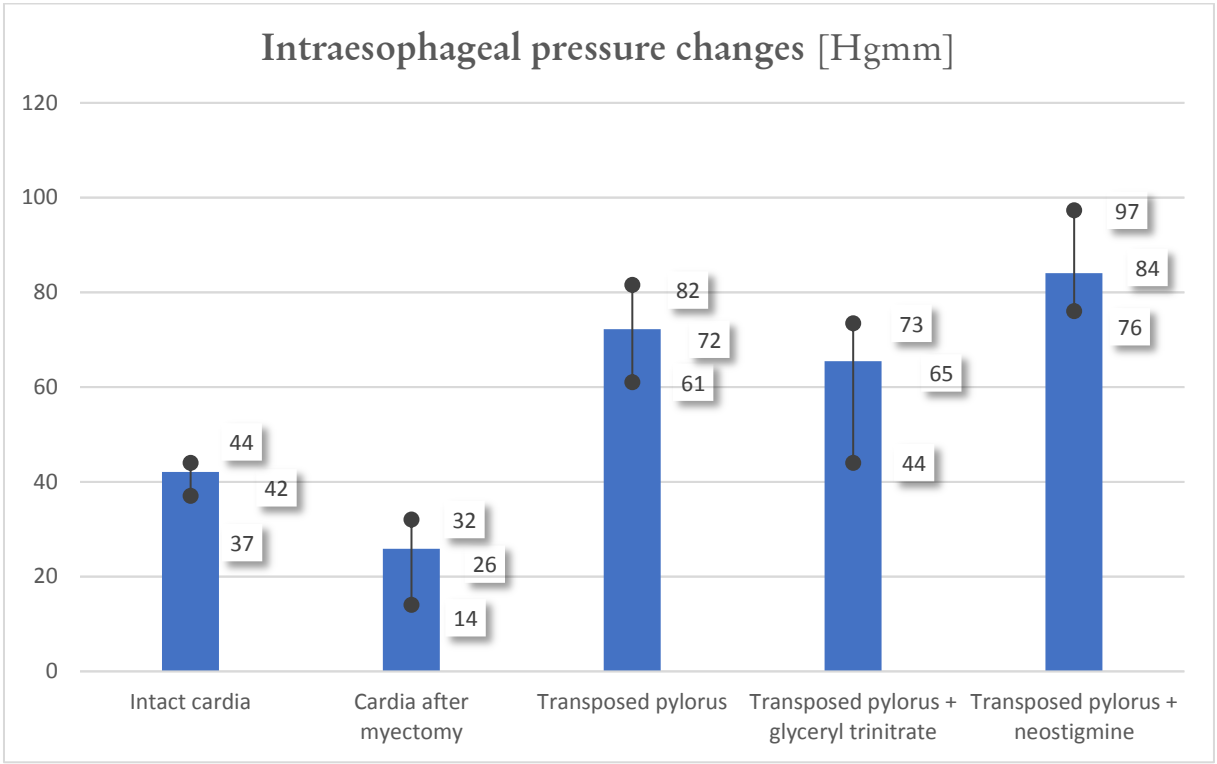

Figure 16. Changes in the intraesophageal pressures measured by manometry (minimum, mean and maximum pressure values in *mmHg*)

The continent ileostomy model was also technically feasible, the left gastroepiploic arterial pedicle was sufficiently long to reach the ileostomy *(Figure 17)*. These two porcine models were surviving animals. On the second postoperative day, the animals were re-operated, the surgical field was reviewed. We found that the gastroepiploic arterial pedicle was well pulsating, the pyloric ring was viable and caused no ischaemia on the bowel.

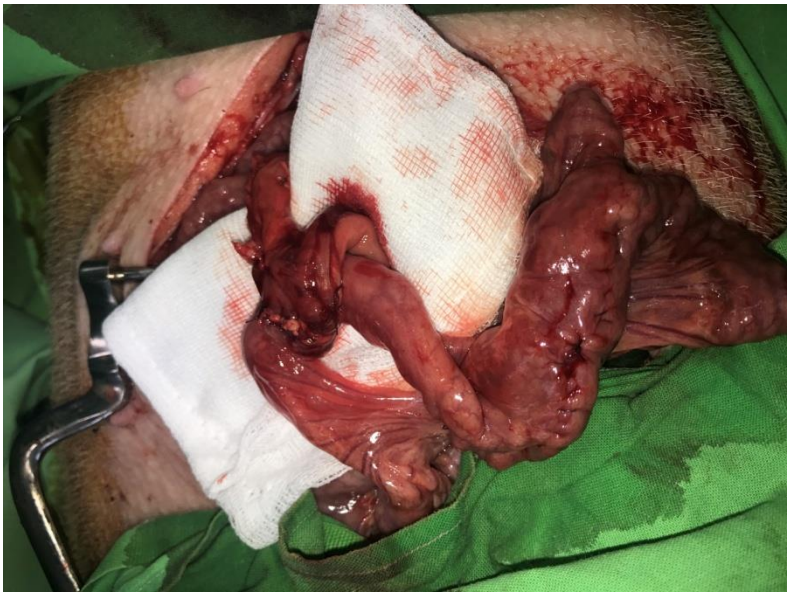

Figure 17. Pedicled pyloric sphincter transposed around the efferent loop of an S-pouch

2.4 DISCUSSION

The left and right gastroepiploic arteries run along the greater curvature of the stomach. In humans, the left and right GEA form a continuous arcade in 70% of the cases [7]. Various uses of the arcade are known in reconstructive surgery. Blood supply to a reconstructed gastric tube after esophagectomy is mainly through the right gastroepiploic artery. Or, the right GEA has been, for example, recognized as a suitable and reliable conduit for coronary bypass surgery, with excellent clinical results [8]. Harvest of omental flaps based on the left or right gastroepiploic artery and vein has proved to be a valid alternative for the coverage and treatment of mediastinal or sternal wounds [9, 10] and also in patients with head and neck defects [11]. Similarly, several authors reported that the use of laparoscopically harvested pedicled omental flaps is a safe and feasible reconstructive option in oncoplastic breast surgery with good cosmetic outcomes [12].

The use of different autologous tissues in reconstructive surgery has always roused the fantasy of surgeons, as implantation of foreign materials may always be the source of complications. In antireflux surgery, the two most commonly chosen procedures are total (Nissen 360°) and partial [anterior (Dor 180°) or posterior (Toupet 270°)] fundoplications. If the defective lower esophageal sphincter is associated with a hiatal hernia, fundoplication has to be completed with a hiatoplasty. Previously we introduced new biologic reinforcement options in humans for large hiatal closures: one with the use of ligamentum teres, the other with the use of fascia lata grafts [13, 14]. Both techniques proved to be feasible, safe and promising options for the treatment of large hiatal hernias. For solitary defective lower esophageal sphincters fundoplication is the gold standard surgical treatment, though its popularity has somewhat declined due to concerns regarding wrap durability and adverse events. Especially after Nissen fundoplication, early postoperative complications, such as dysphagia, bloating or diarrhea, can occur in 50–70% of patients, however, these are usually solved by themselves in 3–6 months [5]. Recurrence of reflux in the late postoperative period occurs in 15–30% in the literature, which may in some cases necessitate reoperation [15]. In the Department of Surgery, University of Pécs, between 1998 and 2015, our surgical team operated on a total of 407 patients with GERD, all of whom underwent laparoscopic – mainly total – fundoplication [16]. Reoperation was necessary in 50 patients (12%), mostly due to postoperative dysphagia and wrap migration. These complications aspired us to search for an alternative, new antireflux procedure that may possibly eliminate these undesirable complications and side-effects. Gaining confidence from the publications on the successful transfer of the pyloric sphincter into the anal region, we got the idea to use a pedicled pyloric sphincter to restore the high pressure zone of a weakened lower esophageal sphincter. We chose the left GEA as pedicle for the pylorus, but the right GEA pedicle would have also reached the lower esophagus. During the experiment, the use of tracheostomy instead of conventional intubation was favourable, since by so doing, changes around the esophagus and cardia could be examined undisturbed. Our reflux animal model [17] was not perfect though, as we intentionally did not extend the circular myectomy to the whole gastroesophageal junction. The reason for this is that excision of all muscle fibers in this region is very difficult and we wanted to avoid any damage to the mucosal tube. Consequently, after the limited my-

ectomy we didn't completely ablate the pressure in the gastroesophageal junction, it only decreased from 42 mmHg to 26 mmHg. The cut pyloric ring was sutured around the impaired gastroesophageal junction at its original diameter. Manometry then showed that the distal intraesophageal pressure increased from 26 mmHg to 72 mmHg, meaning that the pyloric ring is a too tight structure to restore an antireflux barrier and the animal would very likely experience difficulty swallowing in the future. So we concluded that, however, the operation itself was technically feasible, the pyloric sphincter would not be suitable to replace a damaged lower esophageal sphincter due to its narrow lumen and high resting tone, and in this location, it would likely cause mechanical obstruction. Regardless of this finding, we continued the experiment to explore the response of the ring to different drugs. Injection of *neostigmine* caused contraction in both the smooth muscle of the ring and in its vessels. In contrast to this, a bolus of intravenous *glyceryl trinitrate* resulted in a rise in the microcirculation, and in the relaxation of the ring. However, we have to note that these measured changes in the pressure values could be partly attributed to the spared muscle fibers of the gastroesophageal junction, as they evidently also responded to the injected medicines. These findings provided evidence that the cut and resutured pedicled pyloric sphincter responded to drugs in the new, ectopic position with muscle contraction and relaxation and with changes in the microcirculation. This effect met our expectations, since in our second series of experiment, as a preliminary study, we aimed to create a completely continent ileostomy by suturing the pyloric sphincter as a constricting force around the efferent loop of the ileal reservoir and thus providing constant continence, which could then be intentionally opened by controlled pharmacological relaxation of the pedicled pyloric ring. Despite its name, the continent ileostomies we create these days are not completely continent. Kock pouch is an appropriate surgical approach today for selected patients, who are not candidates for ileal pouch anal anastomosis. However, the technique of pouch construction, especially of the valve is complex and is associated with a high incidence of complications and reoperation [6, 18]. In order to eliminate the valve-related problems, in our experiment we replaced the valve itself with a pyloric sphincter based on the left gastroepiploic artery. In case that the left and right GEA do not form an arcade (30% of the cases), the pyloric ring could be based on the right GEA as well. In these cases the arterial pedicle will be shorter, but with appropriate

planning of the ileostomy site, the operation can be technically feasible. The diameter of the pyloric ring could be calibrated to the diameter of the bowel, with care taken to avoid ischaemization of the intestine. The operation was technically feasible, and at reoperation on the second postoperative day, we found that the pyloric ring was viable and caused no ischaemia on the bowel. An ileostomy of this kind could offer patients constant continency without facing the valve related complications of the S-pouch. Normally, anal resting tone varies from 40 to 70 mmHg [19]. With adequate intraoperative calibration, these pressure values could be reached with the transferred pyloric sphincter. It is indisputable that this technique will not allow the pouch either to drain itself deliberately and patients will have to intubate their reservoirs for evacuation in the future too, but avoiding complications related to valve dysfunction could be considered as an advantage.

CHAPTER 3

PROGNOSTIC ROLE OF HPV INFECTION IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

3.1 INTRODUCTION

Esophageal cancer is the eighth most common malignant tumor and the sixth leading cause of cancer mortality worldwide, with approximately 500,000 new cases diagnosed and an estimated 406,000 deaths each year [1]. Esophageal cancer has a poor prognosis with a 5-year survival rate around 15–20%, mainly due to the absence of early symptoms and therefore late stage diagnosis [2]. Despite increasing rates of esophageal adenocarcinoma in many western countries, esophageal squamous cell carcinoma (ESCC) remains the dominant histological type of esophageal cancer globally. The incidence of squamous cell carcinoma of the esophagus varies considerably from place to place, suggesting an important role of environmental factors in its etiology [3]. The main risk factors involved in the etiology of the disease are well established, including alcohol and tobacco consumption and low socioeconomic status. The hypothesis that HPV could potentially be involved in the pathogenesis of esophageal malignancies was first proposed by Syrjänen *et al.* in 1982 [4]. Since then, the connection between HPV infection and esophageal squamous cell carcinoma has been widely studied. Several systematic reviews and meta-analyses have been published recently that observed a close association between HPV infection and the incidence of ESCC [5, 6, 7, 8]. However, the presumed underlying oncogenic mechanisms of HPV-induced esophageal squamous cell carcinoma are poorly understood, and until now, the International Agency on Research on Cancer has not made a definite statement on the potential etiologic relationship between HPV and ESCC.

The impact of HPV infection on response to the oncological treatment and survival is not fully elucidated yet. In locally advanced esophageal cancer neoadjuvant chemoradiotherapy (CRT) can downsize the primary lesion, decrease the potential for metastasis, increase the resectability rate and consequently improve long-term survival [9, 10]. It is

well known, that patients with locally advanced esophageal cancer respond differently to neoadjuvant therapy, due to unexplained factors. In a previous study we found that with neoadjuvant CRT complete pathological response was achieved in 17% of patients and, partial response in approximately the half, while in 16% of the cases stable disease and in 15% progression was observed [11]. Significant improvement in long-term survival can only be expected in patients who have complete pathological response, emphasizing the need for finding prognostic markers that can distinguish between the responder and non-responder group, and consequently save non-responder patients from unnecessary over-treatment with cytostatics. So far no clinically relevant markers have been found that could predict the response to preoperative therapy. Expression levels of stress-inducible heat shock proteins (Hsp) are well-known to be altered during malignant transformation, either increasing or decreasing [12], and studies have also shown that the expression of heat shock proteins is closely related to the prognosis of carcinomas [13, 14]. However, limited and inconsistent reports exist on the relationship between Hsp expression and response to neoadjuvant chemoradiotherapy in ESCC patients. Furthermore, to our knowledge, there have been no studies investigating the association between HPV infection and heat shock protein expression patterns in ESCC patients. Similarly, growth hormone-releasing hormone receptors (GHRH-R) have been found in a variety of tumoral tissues and cell lines and their expression levels proved to be an independent predictor of patient prognosis [15].

The present study aimed to evaluate the effect of tumor HPV status on the prognosis and response to CRT in patients with ESCC. It was also our goal to investigate the correlation between the expressions of Hsp-s (90, 27, 16.2), GHRH-R and response to therapy and overall survival.

3.2 PATIENTS AND METHODS

3.2.1 Patients

A retrospective histological examination of pre-treatment tumor tissue samples from patients with locally advanced esophageal squamous cell carcinoma was carried out. All patients received chemoradiotherapy at the Department of Oncotherapy, Clinical Center,

University of Pécs, Hungary, between 2006 and 2016. Following oncological treatment, patients either underwent surgery or continued chemoradiotherapy. Inclusion criteria required that all examinations and treatments of the patients had to be carried out in the Clinical Center of the University of Pécs. 80 patients were originally enrolled in the study, 6 patients were subsequently excluded for different reasons, leaving us with 74 valid patients. 65 of the 74 patients were active smokers. Regarding sex ratio a high male dominance was observed (58 males:16 females). 12 patients had upper third, 41 patients had middle third and 21 patients had lower third esophageal tumor. All patients had squamous cell cancer, with stages cT3-4, cN0-2, cM0-1. The staging procedure included endoscopy, endoscopic ultrasound, chest X-ray, computed tomography (CT) and bronchoscopy with brush cytology. As oncological treatment, patients received CT planned external-beam radiotherapy (180 cGy daily for 5 days weekly up to 39.6–45 Gy) and concomitant chemotherapy during the first week of irradiation: cisplatin (60–100 mg/m^2) on day 1, 5-fluorouracil (750–1000 mg/m^2/day) and Ca-folinat (20 mg/m^2/day) infusions on days 1–5. After a six-week-long treatment-free period, restaging was carried out according to the Response Evaluation Criteria in Solid Tumors (RECIST) [16]. In order to simplify the evaluation of the results, patients were divided into two groups: *responders* including patients who showed complete or partial response and *non-responders* including patients where either stable disease or disease progression were observed.

3.2.2 HPV detection

Sections from the pre-treatment tumor tissue samples were fixed in formalin and embedded in paraffin. The presence of HPV was detected by chromogenic in situ hybridization (CISH) using ZytoFast PLUS Implementation Kits. Briefly, this system detects HPV types 6, 11, 16, 18, 31, 33, 35, 45, 51 and 82 using Digoxigenin-labeled probes which are detected using primary antibodies. These antibodies are then detected by polymerized enzyme-conjugated secondary antibodies. The enzymatic reaction of chromogenic substrates leads to the formation of strong color precipitates that can be visualized by light microscopy.

Tissue samples that were positive for HPV by CISH were subsequently genotyped using the Linear Array HPV Genotyping Test (Cat. No: 04391853190, Roche Diagnostics,

Mannheim, Germany) according to the accredited molecular biological routine method in the Molecular Genetic Laboratory of the Department of Laboratory Medicine, University of Pécs (accreditation number: NAH molecular biology diagnostics L7-1 MLMB01, Roche Linear Array HPV Genotyping Test). This test can simultaneously detect up to 37 different HPV genotypes in one sample.

3.2.3 Immunohistochemical staining for Hsp 90, 27 and 16.2 and GHRH-R

Immunohistochemical reactions were carried out by LEICA BOND automated staining machines, using polyclonal rabbit antibodies directed against the human Hsp 90, 27, 16.2 and GHRH-R. The formalin-fixed, paraffin-embedded tumor biopsies were sliced into 4 μm thick sections and dried in a 56 C thermostat for 2 hours. The tissue sections were deparaffinized with Bond Dewax Solution, for 8 minutes. Antigen retrieval was carried out with a Bond Epitope Retrieval Solution for 20 minutes, at 97 C and pH 6.00. The conditions were identical for each of the 4 antibodies. The specimens were then incubated with the primary antibodies at 42 C, for 15 minutes, according to the following dilutions: Hsp 90 – 1:100; Hsp 27 – 1:4000; Hsp 16.2 – 1:1000; GHRH-R: 1:50. The sections were then incubated with the secondary antibodies at 42 C, for 10 min, without dilution and they were counterstained with hematoxylin and eosin. The immunoreactions were visualized by Bond Polymer Refine Detection. The tissue sections were dehydrated through an ascending series of alcohol, then covered in xylene and Pertox.

The immunostaining was interpreted by our pathologist blindly, without knowledge of the treatment response rate of the patients. The presence of cytoplasmic staining with or without nuclear staining was required to assign the positivity for heat shock proteins and GHRH-R. Staining intensity was sorted into three categories: 1. samples that showed homogenous strong expression of the antigens 2. samples that showed heterogenous staining pattern with typically weaker staining intensity 3. samples that showed no staining at all. For easier evaluation these categories were then simplified into two classes: *high-intensity* (1) and *low-intensity* (2+3) samples.

3.2.4 Statistical analysis

Finally, the correlations between the HPV status and response, and between the HPV status and the different biomarkers were established with the Chi-Square test. Survival rates of the HPV positive and negative groups were estimated using the Kaplan-Meier method, and compared with the log-rank test. A *p-value*<0.05 was considered significant for the comparison. For the statistical analysis the IBM SPSS Statistics v23 software package was used.

3.3 RESULTS

3.3.1 Clinical and patient data

Of the 74 patients participating in the study, 22 patients (30%) received neoadjuvant CRT and 52 patients (70%) received definitive CRT due to their general condition and/or advanced stage of the disease. 38 patients (51%) responded well to therapy. Ultimately, 14 out of the 22 patients, who had neoadjuvant CRT, underwent surgical resection. Reasons for not having surgery included: not responding well to CRT (5 patients), refusing to consent to surgery (2 patient) and death (1 patient). 14 (19%) of the 74 ESCC patients were found to be HPV positive with CISH *(Figure 18)*.

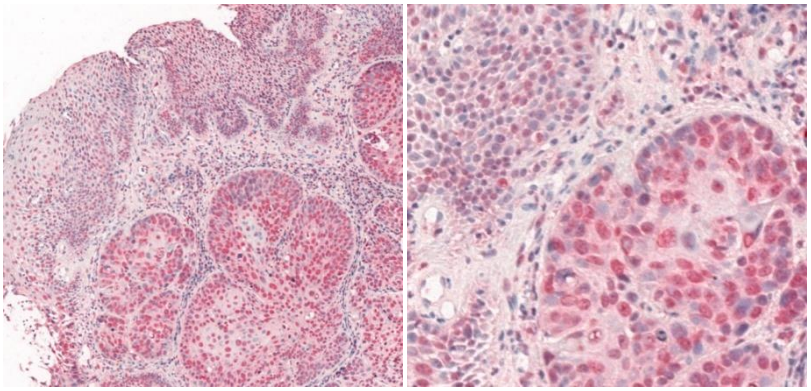

Figure 18. HPV positivity determined with CISH

Regarding the distribution of the sexes and locations of the tumors, in the HPV positive group we found a male:female ratio of 8:6, where 4 patients had upper third,

6 patients had middle third and 4 patients had lower third esophageal tumor, while in the HPV negative group the male:female ratio was 5:1, and among them 8 patients had upper third, 35 had middle third and 17 had lower esophageal tumor. Baseline characteristics of HPV-positive and HPV-negative patients are shown in *Table 4*.

Table 4. Characteristics of HPV negative versus HPV positive patients

Variable		**HPV negative**	**HPV positive**
		(*n=60*) no. (%)	(*n=14*) no. (%)
Age at diagnosis	≤ 60	29 (48.3%)	4 (28.6%)
	>60	31 (51.7%)	10 (71.4%)
Gender	Female	10 (16.7%)	6 (42.9%)
	Male	50 (83.3%)	8 (57.1%)
Tumor location	Upper third	8 (13.3%)	4 (28.6%)
	Middle third	35 (58.3%)	6 (42.8%)
	Lower third	17 (28.4%)	4 (28.6%)
Clinical T stage	cT3	26 (43.3%)	8 (57.1%)
	cT4	34 (56.7%)	6 (42.9%)
Clinical N stage	cN0	6 (10.0%)	4 (28.6%)
	cN1–2	54 (90.0%)	10 (71.4%)
Clinical M stage	cM0	49 (81.7%)	14 (100.0%)
	cM1	11 (18.3%)	0 (0.0%)

We also aimed to detect HPV DNA sequence in our positive cases to examine the distribution of HPV genotypes. Unfortunately, repeated linear array tests failed to give results for proper laboratory evaluation. This might be due to overfixation of the small tissue samples in formaldehyde, which resulted in the damage and degradation of the DNA.

3.3.2 Effects of HPV status on response to therapy and prognosis

Comparing the HPV status and the clinical response to CRT, we found that HPV positivity was associated with a higher rate of non-responder patients (71.4% non-responders vs. 28.6% responders), however, this difference was not significant (Chi-Square $p=0.058$) *(Table 5)*.

Table 5. The effect of HPV status on response to CRT

		Clinical Downstaging (*n*=74)		*p value*
		Responder	**Non-responder**	
HPV	**negative**	34 (56.7%)	26 (43.3%)	*p=0.058*
	positive	4 (28.6%)	10 (71.4%)	

Similarly, the overall survival of HPV-positive patients was shorter compared to HPV-negative patients (mean survival of 8 months vs. 11 months and median survival of 6 months vs. 7 months), but this difference was also not significant (log-rank p=0.898) *(Figure 19)*.

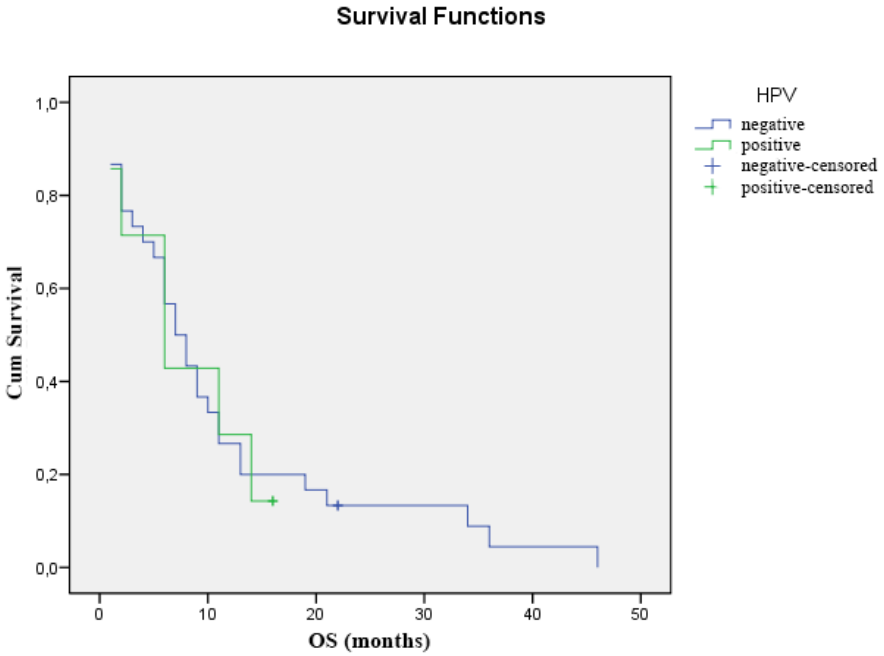

Figure 19. The effect of HPV status on overall survival demonstrated with Kaplan-Meier curve.

3.3.3 Relationship between HPV status and expressions of Hsp 16.2, 27, 90 and GHRH-R

Significantly more HPV positive tumors expressed Hsp 90 and 16.2 at high intensities than at low intensities (Chi-Square p=0.019 and p=0.031). On the other hand, there was a near-equal distribution of low and high intensity Hsp staining in HPV negative tumors.

No significant correlation could be observed between the Hsp 27 and GHRH-R expression patterns and HPV positivity *(Table 6)*.

Table 6. The relationship between HPV status and expressions of Hsp 16.2, 27, 90 and GHRH-R

Molecular Marker		**HPV status** ($n=74$)		*p value*
		negative	**positive**	
Hsp 16.2	low intensity	32 (53.3%)	3 (21.4%)	*p=0.031*
	high intensity	28 (46.7%)	11 (78.6%)	
Hsp 27	low intensity	30 (50.0%)	6 (42.9%)	*p=0.630*
	high intensity	30 (50.0%)	8 (57.1%)	
Hsp 90	low intensity	24 (40.0%)	1 (7.1%)	*p=0.019*
	high intensity	36 (60.0%)	13 (92.9%)	
GHRH-R	low intensity	42 (70.0%)	8 (57.1%)	*p=0.355*
	high intensity	18 (30.0%)	6 (42.9%)	

3.3.4 The effects of Hsp expression on response to therapy

Among non-responders, there were significantly more tumors, which expressed Hsp 90 and 16.2 at high levels (Chi-Square $p<0.001$ and $p<0.01$). This tendency was also apparent in the expression levels of Hsp 27, but the difference was not significant *(Table 7)*. We also found that patients with tumors that expressed Hsp-s at high levels had a significantly shorter overall survival, than patients with tumors that stained low for Hsp-s. *(Figures 20 and 21)*.

Table 7. The effects of Hsp expression on response to CRT

Molecular Marker		**Clinical Downstaging** (n=74)		*p value*
		Responder	**Non-Responder**	
Hsp 16.2	low intensity	29 (82.9%)	6 (17.1%)	*p<0.01*
	high intensity	9 (23.1%)	30 (76.9%)	
Hsp 27	low intensity	22 (61.1%)	14 (38.9%)	*p=0.102*
	high intensity	16 (42.1%)	22 (57.9%)	
Hsp 90	low intensity	21 (84.0%)	4 (16.0%)	*p<0.001*
	high intensity	17 (34.7%)	32 (65.3%)	

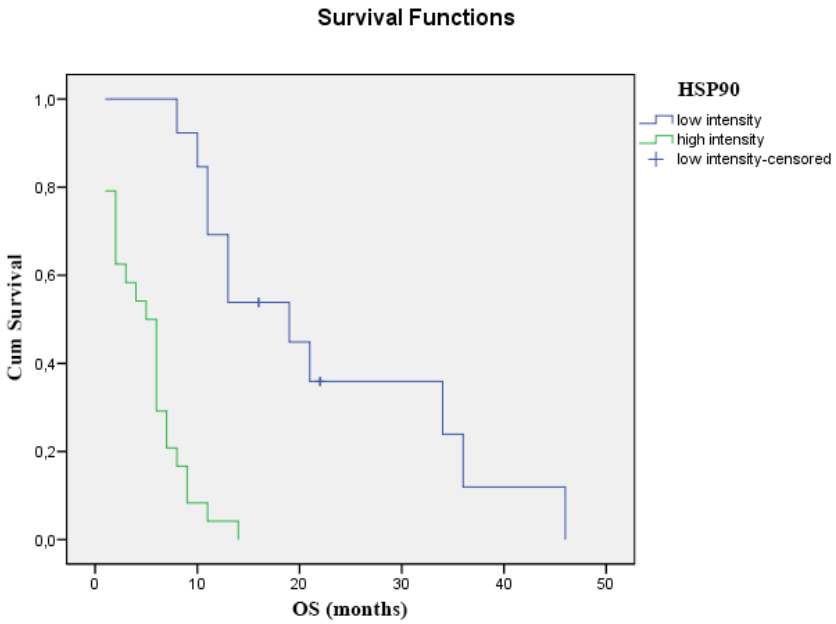

Figure 20. The effect of Hsp 90 expression on overall survival is demonstrated using Kaplan-Meier curve and the level of significance is determined using the log-rank test. Probability (p) values <0.05 are considered statistically significant. p<0.001.

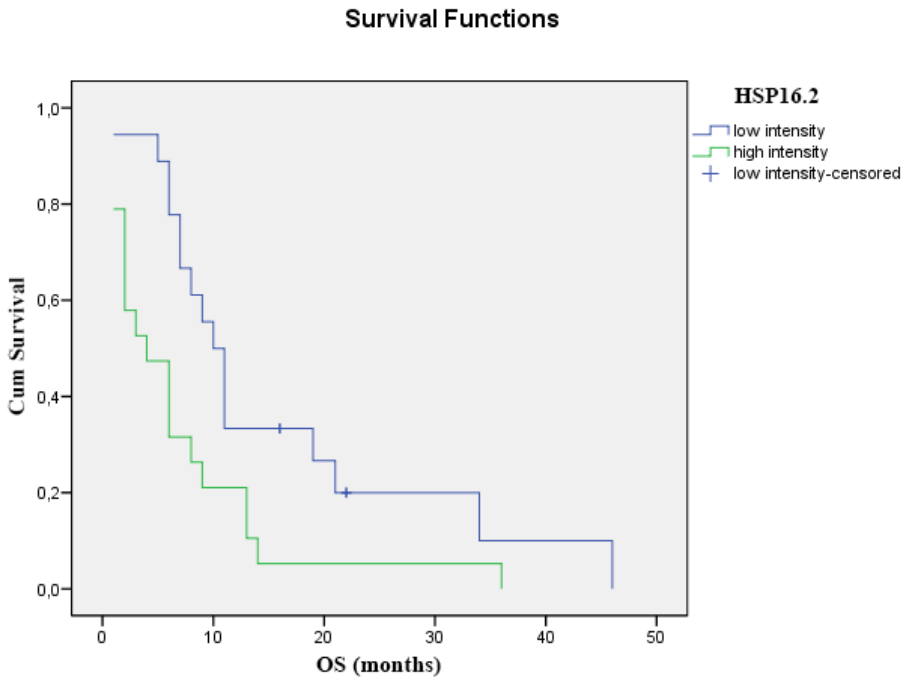

Figure 21. The effect of 16.2 expression on overall survival is demonstrated using Kaplan-Meier curves and the level of significance is determined using the log-rank test. Probability (p) values <0.05 are considered statistically significant. p<0.001.

3.4 DISCUSSION

In recent years, a large number of studies have investigated human papillomavirus infection in esophageal squamous cell carcinoma, with largely inconclusive results [5, 6, 7, 8]. The detection rates of HPV-ESCC show high variability worldwide. There are large geographic differences in the overall incidence of ESCC, with high-incidence countries within the 'Asian esophageal cancer belt' reporting up to one-hundred-fold higher rates of ESCC compared to low-incidence countries, such as the United States, Europe or Australia [17]. In high-ESCC-incidence countries, the HPV detection rate in tumor tissues is also significantly higher compared to low-ESCC-incidence countries (32.8-63.6% in China vs. 8.7–16.6% in North America) [18]. In our study, among the Hungarian population, the rate of HPV positivity in ESCC patients corresponded to that of the low risk countries. Namely, 14 (19%) out of the 74 patients were confirmed to be HPV positive by CISH. The prognostic value of HPV status has previously been investigated in patients with ESCC [19, 20, 21]. The first meta-analysis investigating overall survival in HPV-related esophageal cancer was published in 2016, and showed no significant associa-

tion between HPV infection and survival [22]. The controversial results of the included studies are in contrast to oropharyngeal lesions, where HPV-positivity has been consistently shown to be a strong positive prognostic factor in patient outcomes [23, 24]. A potential correlation between HPV infection and response to neoadjuvant treatment in patients with ESSC has also been the subject of ongoing debate. Several studies demonstrated that HPV-positive cervical cancer patients had significantly better clinical response to oncological treatment and survived significantly longer than HPV negative patients [25, 26]. Therefore, HPV can be considered an independent prognostic parameter for radiosensitivity and survival in patients with cervical cancer. HPV infection in esophageal cancer as a possible predictive factor before neoadjuvant therapy has been studied before [27, 28]. Bognár *et al.* and Wang *et al.* found a correlation between HPV infection and response to CRT. They both reported that HPV positive patients responded better to CRT and had a significantly more favorable survival compared to the HPV negative group; however, due to the relatively low number of patients involved, far-reaching conclusions could not be drawn. In our study, we found an opposite result, namely that the HPV-positive group responded worse to CRT and had worse overall survival than the HPV-negative group. Therefore, in our study, HPV positivity was a negative prognostic factor in relation to multimodal therapy and to overall survival, though the differences were not significant. In our study we also examined the anti-apoptotic Hsp 90, 27 and 16.2 expression patterns in the pre-treatment tumor biopsies. Heat shock proteins are induced in response to a wide variety of physiological and environmental insults, thus allowing cells to survive lethal conditions based on their cytoprotective functions. Associated with key apoptotic factors, they are powerful anti-apoptotic proteins, having the capacity to block cell death process at different levels. Hsp overexpression signals a poor prognosis in terms of survival and response to therapy in specific cancer types [29, 30]. In a previous study, we examined the Hsp 90 and 16.2 expression of esophageal tumor specimens prior to CRT, in search of possible predictive biomarkers of response to multimodal therapy [31]. We found that the tumor samples from the patients with no clinical response contained approximately double the level of Hsp 90 and 16.2, significantly higher than the responding tumors. In the present study, we examined whether HPV infection, as an environmental insult, influences the Hsp expression pattern of ESCC patients. We

found elevated Hsp 90 and 16.2 expression levels in the HPV-positive tumor samples compared to the HPV-negative ones. As expected, increased levels of Hsp 90 and 16.2 expression, were associated with significantly poorer response to CRT and worse overall survival. It is unclear why HPV positivity in ESCC patients proves to be a negative prognostic marker in certain regions of the world, while in others it is a positive prognostic marker. In head and neck tumors, the development of cancer is attributed to different oncogene mechanisms in patients with HPV positive tumors and in those who don't carry the virus but have a dominant history of alcohol and tobacco consumption [32]. The biology of HPV-positive oropharyngeal cancer is characterized by p53 degradation, retinoblastoma Rb pathway inactivation, and p16 upregulation, while, by contrast, tobacco-related oropharyngeal cancer is characterized by TP53 mutation and downregulation of CDKN2A (encoding p16) [33]. It is also well known that HPV-positive oropharyngeal cancers seem to be more responsive to chemotherapy and radiation than HPV-negative tumors. In line with these findings, we have set up a hypothesis, which could explain why HPV-positive esophageal squamous cell cancers respond differently to multimodal therapy. In regions where the HPV detection rate in esophageal tumors is high, a positive correlation can be observed between HPV positivity and response to treatment. We presume that in these cases the viral infection plays a role in the cancerogenesis itself, while in the low-risk regions, the development of cancer is attributed to other factors, such as poor socioeconomic environment, excessive alcohol and tobacco consumption, and HPV only superinfects the esophagus. The evaluation of p16 expression, a surrogate biomarker for HPV infection, is also of importance regarding prognosis of ESCC. Expression of p16 in ESCC means an active HPV infection in tumor cells and has been shown to correlate with higher rate of pathologic complete remission in patients undergoing neoadjuvant chemotherapy [34], compared with p16 negative individuals, who carry HPV DNA. We presume that this may be attributed to that in p16 positive individuals the virus itself induced the cancerogenesis, while in p16 negative cases that were HPV DNA positive, HPV means only a superinfection of the tumorous cells. This superinfection, as an environmental insult, could lead to an increased expression of heat shock proteins, and as a consequence these tumors respond worse to anticancer treatments. This hypothesis brings up an unanswered issue of the current understanding of the epidemiology and bi-

ology of HPV-associated esophageal squamous cell carcinoma. Evaluation of p16 expression besides HPV DNA status would be thus imperative in pre-treatment ESCC samples to differentiate between an active and a passenger HPV infection and consequent, potentially different prognosis. Unfortunately, in our study analysis of p16 expression could not be undertaken due to the degradation of the tissue samples, leading to major limitation of our study. As such, to confirm this hypothesis further studies are needed.

Today, the Advisory Committee on Immunization Practices (ACIP) recommends routine HPV vaccination for females and males at age 11 or 12 years, to prevent infection with HPV types that are associated with certain cancers, including cervical, vaginal, vulvar, anal, throat and penile cancers [35]. The recommendation doesn't comprise the prevention of HPV-associated esophageal cancers, however, growing literature demonstrates that the virus is involved in the development of esophageal squamous cell carcinoma or may worsen the prognosis. In our opinion, extension of the indications of prophylactic immunization is imperative.

Growth hormone-releasing hormone (GHRH) is a peptide hormone secreted by the hypothalamus, but it is also present in various tissues and tumors, stimulating the secretion of growth hormone (GH) after binding to pituitary-type GHRH receptors (GHRH-R) on the anterior pituitary. GH stimulates the production of the insulin-like growth factor I (IGF-I), which plays a major role in malignant transformation, metastasis and tumorigenesis in various cancers [36]. The presence of GHRH-R and its splice variants, on different types of cancer cell lines has been demonstrated [37-39]. In our study, we found association neither between GHRH-R expression and the HPV status, nor between GHRH-R expression and response to treatment.

In conclusion, the present study found that one-fifth of the patients with ESCC proved to have HPV-positive tumors in Hungary's Southwestern region. HPV positivity was accompanied by significantly increased expressions of Hsp 90 and 16.2. HPV-positive cases and cases expressing high intensity Hsp 90 and 16.2 levels showed a significantly poorer response to oncological treatment and worse overall survival. We admit the limitations of our study. Given the limited sample size, the results of this report should be interpreted with caution. To confirm the significance of our observation further larger scale studies are needed.

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NOVEL FINDINGS

ESOPHAGEAL COMPLICATIONS OF GASTROESOPHAGEAL REFLUX DISEASE: CONSEQUENCES OR DEFENSIVE REACTIONS?

1 This study was the first to discuss gastroesophageal reflux disease as a potential causative factor in the development of nine different esophageal and one airway disorders that result in decreased reflux symptoms, reduced acid regurgitation, and through this they reduce the risk of aspiration as well. We concluded that the functional esophageal changes (HLES, HUES, achalasia, diffuse esophageal spasm) that develop following long-standing GERD are adaptive reactions, aimed at easing the unpleasant reflux symptoms and reducing acid regurgitation. The development of Barrett's esophagus can also be regarded as an adaptive change, as it is associated with a downturn in reflux symptoms. The structural changes (Schatzki's ring, esophageal web, esophageal stricture, subglottic tracheal stenosis) also result in reduced acid regurgitation, but we consider that these are rather secondary consequences of GERD and not real adaptive mechanisms.

2 Based on our experiences and review of the literature we assume that in those functional esophageal disorders, where the etiological role of GERD arises, therapeutic strategy should be different from that of the same, but primary esophageal motility disorder. In all of these cases, therapy should focus on the treatment of GERD. Our recommendation is as what follows in case of GERD induced

- HLES: laparoscopic total (Nissen 360°) fundoplication
  (not recommended: myotomy of the LES + fundoplication)
- HUES: dilation therapy and long-term treatment with acid suppression medication
  (not recommended: myotomy of the UES)
- Zenker's diverticulum: surgical or endoscopic treatment of the diverticulum + cricopharyngeal myotomy + long-term treatment with acid suppression medication
- achalasia: laparoscopic Heller's myotomy and total (Nissen 360°) fundoplication

(not recommended: ballon dilation)

- DES: long-term treatment with acid suppression medication
  (not recommended: use of smooth muscle relaxants)

FUNCTIONAL EXAMINATION OF THE TRANSPOSED PYLORIC SPHINCTER IN ANTIREFLUX PORCINE MODELS: COULD IT BE SUITABLE TO CREATE A CONTINENT ILEOSTOMY?

3 Transposition of a pedicled pyloric sphincter around the impaired gastroesophageal junction is technically feasible, however, it cannot be recommended for antireflux procedure due to the narrow lumen and high resting tone of the pyloric ring, which would lead to dysphagia in this location.

4 Our animal study proved evidence that the pedicled pyloric ring preserved its pharmacological responsiveness in the ectopic position. Injection of *neostigmine* resulted in contraction in both the smooth muscles of the ring and in its vessels. Injection of *glyceryl trinitrate* resulted in relaxation of the ring and in an increase in the microcirculation of the ring.

5 In our preliminary study, our novel approach to create a continent ileostomy with the use of a pyloric sphincter based on the left gastroepiploic artery proved to be technically feasible. Planned reoperation revealed that the ring did not cause ischaemia on the small intestine.

PROGNOSTIC ROLE OF HPV INFECTION IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

6 In our study, the rate of HPV positivity in Hungarian esophageal squamous cell cancer patients corresponded to that of the low risk countries, namely, 19% of the patients were confirmed to be HPV positive by CISH.

7 In our study, the distribution of HPV positivity in the upper, middle and lower thirds of the esophagus were 28,6%, 42,8% and 28,6%, respectively. Thus, upper third cancers did not harbour higher rates of HPV, as it could have been expected due to the proximity of the oral cavity.

8 In our study, we found that HPV infection was associated with worse response to oncological treatment and decreased overall survival, and therefore proved to be a negative prognostic factor in patients with esophageal squamous cell carcinoma.

9 In our study, we found that HPV positivity was associated with high expression levels of Hsp 90 and 16.2. High expression levels of Hsp 90 and 16.2 were associated with worse prognosis. We found association neither between Hsp 27 or GHRH-R expression and the HPV status, nor between Hsp 27 or GHRH-R expression and response to treatment.

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CUMULATIVE IMPACT FACTOR OF ALL PUBLICATIONS: 14.546

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