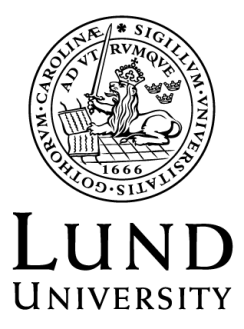
Posterior Laryngitis

Aetiology, Treatment and Health-Related Quality of Life    
  
  
by

Hillevi Pendleton



AKADEMISK AVHANDLING

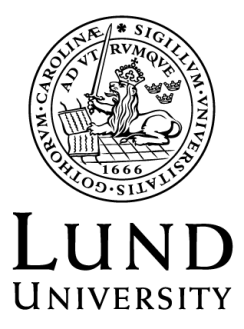
som för avläggande av doktorsexamen vid medicinska fakulteten, Lunds universitet, kommer offentligen försvaras i Belfragesalen, BMC, Sölvegatan 19, Skånes universitetssjukhus Lund, fredagen den 27 september 2013, kl. 13.00.

Fakultetsopponent

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Posterior Laryngitis

Aetiology, Treatment and Health-Related Quality of Life  





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To Christian, Samuel and Beatrice

and in loving memory of my mother Nancy

who said I could do anything I set my mind to.

# Abstract

Posterior laryngitis (PL) is defined as an inflammation involving the posterior part of the glottal region in conjunction with symptoms. The cause is multifactorial.

The aims of the work presented in this thesis were to examine symptoms, physical signs, different aetiologies and health-related quality of life (HRQOL) in patients with PL and/or oesophageal dysmotility.

In paper I, a group of patients diagnosed as having PL were examined, treated and followed, to determine whether follow-up is needed. Their current HRQOL was registered. In paper II, we investigated how many of the patients diagnosed with PL who had acid reflux in the proximal part of the oesophagus, altered motilin levels, and symptoms associated with functional gastrointestinal disease. Their HRQOL was registered before and after treatment. In paper III, patients with PL were scrutinized for the presence of gonadotropin-releasing hormone (GnRH) antibodies. In paper IV, patients with diabetes mellitus were examined to see whether blood levels of motilin were related to symptoms and signs of dysfunction in the oesophagus and stomach.

Approximately 90% of the patients, investigated for reflux, were treated with proton pump inhibitors. Of the patients not investigated for reflux, 85% received acid-suppressing treatment. One-third of the investigated patients in paper II had objectively measured reflux. Patients with typical reflux symptoms had abnormal levels of motilin compared to those without these symptoms. Antibodies against GnRH and gastrointestinal symptoms were found to a greater extent in patients than in controls. There was a significant correlation between the percentage of simultaneous contractions in the oesophagus, and basic and peak plasma motilin values in patients with diabetes mellitus. HRQOL, especially in women, was low.

Taken together, PL is associated with acid reflux in the proximal oesophagus, endocrine disturbances, functional gastrointestinal disease and reduced HRQOL. By improving follow-up, and optimising investigations and treatment, HRQOL can be improved.

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Paper I: PENDLETON, H., AHLNER-ELMQVIST, M., JANNERT, M. & OHLSSON, B. 2013. Posterior laryngitis: a study of persisting symptoms and health-related quality of life. Eur Arch Otorhinolaryngol, 270, 187-95.

Paper II: PENDLETON, H., AHLNER-ELMQVIST, M., OLSSON, R., THORSSON, O., HAMMAR, O., JANNERT, M. & OHLSSON, B. Posterior laryngitis: a disease with different aetiologies affecting health-related quality of life. BMC Ear Nose Throat Disord, submitted July, 2013.

Paper III: PENDLETON, H., ALM, R., NORDIN FREDRIKSON, G. & OHLSSON, B. 2013. Antibodies against gonadotropin-releasing hormone in patients with posterior laryngitis. Drug Target Insights, 7, 1-8.

Paper IV: PENDLETON, H., EKMAN, R., OLSSON, R., EKBERG, O. & OHLSSON, B. 2009. Motilin concentrations in relation to gastro intestinal dysmotility in diabetes mellitus. Eur J Intern Med, 20, 654-9.

# Abbreviations

CA Carbonic anhydrase isoenzymes

CIPO Chronic intestinal pseudo-obstruction

ED Enteric dysmotility

ENS Enteric nervous system

ELISA Enzyme-linked immunosorbent assay

ENT Ear, nose and throat

FGID Functional gastrointestinal disease

FSH Follicle-stimulating hormone

GIS Gastro-oesophageal reflux disease impact scale

GnRH Gonadotropin-releasing hormone

GORD Gastro-oesophageal reflux disease

HRQOL Health-related quality of life

IBS Irritable bowel syndrome

LH Luteinizing hormone

LOS Lower oesophageal sphincter

LPR Laryngo-pharyngeal reflux

MII-pH Multi-channel intra-luminal impedance and pH monitoring

MMC III Migrating motor complex phase III

OGD Oesophago-gastro-duodenoscopy

PL Posterior laryngitis

PPI Proton pump inhibitor

SF-36 36-item Short-Form questionnaire

VAS-IBS Visual Analogue Scale for Irritable Bowel Syndrome

# Thesis at a glance

Yes

Yes

Do patients with PL have the same endocrine disturbances found in patients suffering from FGID and in patients with diabetes mellitus with gastrointestinal complaints, affecting oesophageal motility?

(paper II, III, IV)

Are there additional aetiologies to PL than LPR and does PL affect HRQOL?

(paper I, II, III)

No

No

Are patients affected by PL adequately investigated and followed-up and is treatment with PPIs effective in this group? (paper I)

Following issues were addressed in this thesis:

Is acidic reflux present in the majority of patients with typical findings and symptoms of PL?

(paper II)

# Introduction

In 1991 an article about oto-laryngologic manifestations of gastro-oesophageal reflux disease (GORD) was published ([Koufman 1991](#_ENREF_81)). The observations made by the author have formed the basis of the knowledge we have about laryngo-pharyngeal reflux-induced (LPR-induced) posterior laryngitis (PL). Reflux of stomach contents into the oesophagus is a common cause of symptoms from the upper gastrointestinal tract. Although the exact mechanism is not known, reflux is associated with oesophageal dysmotility in 50%–60% of the cases ([Kahrilas et al. 1986](#_ENREF_71)) and is also associated with reduced pressure of the lower oesophageal sphincter (LOS) ([Zaninotto et al. 1988](#_ENREF_189); [Kahrilas 1997](#_ENREF_70); [Ho et al. 2002](#_ENREF_57)). Reflux causes heartburn, acid regurgitation and oesophagitis ([Diener et al. 2001](#_ENREF_29); [Ho et al. 2002](#_ENREF_57)). As described by Koufman, reflux may also lead to PL ([Koufman 1991](#_ENREF_81); [Koufman et al. 2000](#_ENREF_82); [Hopkins C 2006](#_ENREF_58)).

Nevertheless, patients with PL suspected to be induced by LPR and treated with acid-reducing medication, are only partly, or not at all improved by medication ([Khan et al. 2006](#_ENREF_77); [Vaezi et al. 2006](#_ENREF_168)). Although several aspects of LPR, and PL as a consequence of LPR have been covered ([Koufman 1991](#_ENREF_81)), other aetiologies for PL or the patient´s health-related quality of life (HRQOL) have gained only scant attention. However, over the past 10–15 years, the impact of PL on HRQOL has begun to be evaluated and aetiologies apart from reflux have been discussed.

When conducting a study within this area it appeared apparent, not only to explore different aetiologies, physical symptoms, and the habits of the patients, but also to try to assess how their HRQOL was affected.

## Posterior laryngitis

Posterior laryngitis is defined as an inflammation involving the most posterior part of the glottic region, sometimes involving the oesophageal inlet in conjunction with symptoms such as chronic cough, hoarseness, a sensation of having a lump in the throat (globus), excessive throat clearing, excessive phlegm, voice fatigue, throat pain and dysphagia ([Koufman et al. 2000](#_ENREF_82); [Hopkins C 2006](#_ENREF_58); [Pearson et al. 2011](#_ENREF_123); [Watson 2011](#_ENREF_178)). In an inspection of the larynx, the physical findings may be laryngeal oedema, erythema of the mucosa over the arytenoid cartilages and/or the vocal chords, pseudo-sulcus formation, and inter-arytenoid thickening ([Powitzky et al. 2003](#_ENREF_132)) (Figure 1).

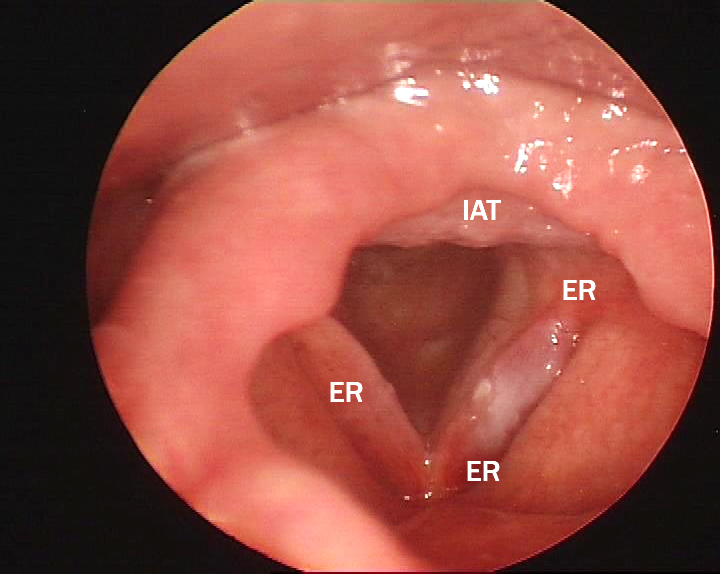


Figure 1. Posterior laryngitis with inter-arytenoid thickening (IAT) and erythema of the mucosa over the arytenoid cartilage and the vocal chords (ER).

The aetiology of inflammation in PL is multifactorial and may be caused by viral or bacterial infections, smoking, alcohol abuse, allergy, chronic sinusitis, voice abuse and LPR ([Kotby et al. 2010](#_ENREF_80); [Pearson et al. 2011](#_ENREF_123)). The main aggressive agents in gastric juice are: acid, pepsin, bile salts, and pancreatic proteolytic enzymes. According to Sataloff et al. ([Sataloff et al. 2010](#_ENREF_137)), epidemiological studies to determine the prevalence of PL and LPR are still lacking.

Four to ten percent of the patients visiting an ear, nose and throat (ENT) specialist have reflux-related complaints in the pharynx and larynx ([Hopkins C 2006](#_ENREF_58)) and of these, as many as 50%–55% may have a pH-documented reflux to the hypopharynx-larynx ([Koufman et al. 2000](#_ENREF_82)).

Bove et al. ([Bove et al. 2000](#_ENREF_14)) have shown that the upper limit for pharyngeal acid exposure in a group of healthy volunteers is 6.1 events/24 h, but at present there is no consensus on the number of reflux episodes to the pharynx that should be considered abnormal. Damage to the pharyngeal mucosa has been observed when as few as two episodes occur in 24 h ([Postma 2006](#_ENREF_129)), while Powitsky et al. ([Powitzky et al. 2003](#_ENREF_132)) have proposed 5 events/24 h as a sign of pathological reflux to the pharynx.

## Gastro-oesophageal reflux disease

The Montreal definition of GORD defines it as *a condition, which develops when reflux of stomach content causes troublesome symptoms and/or complications.* The disease may present itself with symptoms such as heartburn, regurgitations or chest pain, but can also cause oesophageal injury such as oesophagitis, strictures, Barrett´s oesophagus and adenocarcinoma ([Vakil et al. 2006](#_ENREF_169)). Extra-oesophageal symptoms such as chronic aspiration, chronic cough, asthma, and PL are also seen ([Pearson et al. 2011](#_ENREF_123)).

Twenty to thirty percent of the adult population have GORD, making it one of the most common diseases of the gastrointestinal tract ([Pearson et al. 2011](#_ENREF_123)). Rising obesity rates, augmented consumption of medication affecting the lower oesophageal sphincter (LOS), and the frequent use of nicotine and caffeine that lowers the pressure of the LOS could be plausible reasons ([Pandolfino et al. 2008](#_ENREF_117)). GORD is associated with oesophageal dysmotility in 40%–60% of the cases and reduced pressure of the LOS in the majority of the cases ([Zaninotto et al. 1988](#_ENREF_189); [Ho et al. 2002](#_ENREF_57); [Kahrilas et al. 2011](#_ENREF_72)).

### The association between PL and GORD

PL is by some perceived as an extra-oesophageal manifestation of GORD ([Ylitalo et al. 2004](#_ENREF_188); [Watson 2011](#_ENREF_178)), while others consider it as being an entity of its own ([Postma 2006](#_ENREF_129)), and there are several similarities and some differences between the two entities. In one study including only endoscopically proven GORD, a correlation between the severity of GORD and the prevalence of PL was found ([Groome et al. 2007](#_ENREF_46)). Another study could not demonstrate any correlation between severity of oesophagitis and severity of laryngeal findings, and concluded that oesophago-gastro-duodenoscopy (OGD) had no role in the diagnosis of PL ([Vardar et al. 2012](#_ENREF_172)). In studies examining the two separate populations, oesophagitis is more common in GORD than in PL ([Koufman et al. 2002b](#_ENREF_84); [Toros et al. 2009](#_ENREF_164); [Becher and Dent 2011](#_ENREF_8)). Heartburn, which often troubles patients with GORD, is less common in patients who have PL ([Koufman et al. 2002a](#_ENREF_83)). Moreover, those who have PL experience daytime refluxes in the upright position, while reflux is more common in the supine position in GORD ([Postma et al. 2001](#_ENREF_130); [Koufman et al. 2002a](#_ENREF_83)). Powitsky et al. ([Powitzky et al. 2003](#_ENREF_132)) have found that the voice of patients with PL is more affected on a daily basis, than in patients with GORD. Oesophageal dysmotility is present in both patient categories, and seems to be an important factor for how the disease presents itself ([Kahrilas et al. 1986](#_ENREF_71); [Knight et al. 2000](#_ENREF_79)).

### Pathophysiology for LPR and GORD

#### The anti-reflux barrier and mechanisms of reflux

The LOS is a 3–4 cm long high-pressure segment comprised of contracted smooth muscle located at the oesophago-gastric junction ([Pandolfino et al. 2008](#_ENREF_117)). LOS pressure fluctuates during the day and increases during sleep, and it is influenced by numerous factors such as intra-abdominal pressure, extrinsic compression by the crural diaphragm, gastric distension, various foods, intra-luminal peptides, hormones such as motilin and various medications. The crural diaphragm arches around the oesophagus and collaborates with the LOS as a “two sphincter” barrier at the oesophago-gastric junction ([Kahrilas 1997](#_ENREF_70); [Pandolfino et al. 2008](#_ENREF_117)). The abdominal position of the LOS is important for the formation of the flap-valve, a musculo-mucosal fold, called the angle of His, which is created when the oesophagus enters the ventricle. When the intra-abdominal pressure rises, the pressure of the angle of His decreases and the distal part of the oesophagus is compressed, preventing the LOS from opening ([Kahrilas 1997](#_ENREF_70); [Herbella and Patti 2010](#_ENREF_55)).

When the bolus reaches the upper oesophageal sphincter, this relaxes and the bolus can pass to the oesophagus by primary peristalsis. When the bolus reaches the LOS, this relaxes and the bolus reaches the stomach. A second wave of peristalsis, secondary peristalsis, clears the oesophagus from any remaining residues of food or saliva ([Diamant 1990](#_ENREF_28)). Peristaltic dysfunction and oesophageal dysmotility are predisposing factors for reflux, as it compromises oesophageal clearance ([Kahrilas et al., 1986](#_ENREF_62), [Knight et al., 2000](#_ENREF_70)).

Reflux can be caused by different mechanisms influencing the oesophago-gastric junction. Hypotension of the LOS can lead to reflux if the abdominal pressure rises, e.g. with strenuous exercise. Reflux can also occur by transient LOS relaxations, which occur unrelated to swallowing. They are not associated with peristalsis of the oesophagus and last longer than swallow-induced relaxations of the LOS ([Karkos 2011](#_ENREF_75)).

A third mechanism is the anatomical alteration of the oesophago-gastric junction, as for example a hiatal hernia. This reduces the length of the high-pressure zone at the oesophago-gastric junction and compromises the crural diaphragm function. The stomach contents, collected in the hernia, can reflux to the oesophagus with swallowing and thus impair oesophageal clearence ([Pandolfino et al. 2008](#_ENREF_117); [Herbella and Patti 2010](#_ENREF_55)). Although a hiatal hernia is a predisposing factor for GORD, far from all who display a hernia have GORD ([Herbella and Patti 2010](#_ENREF_55)).

#### The mucosal defence

The properties of the mucosa, and the composition of the reflux, may determine the site damaged by reflux. Reflux can be characterised as being acidic with a pH < 4; weakly acidic with a pH between 4 and 7; and weakly alkaline with a pH above 7 ([Arevalo 2011](#_ENREF_5)). The distal oesophageal mucosa has specific defence mechanisms making it resistant to acid exposure. These mechanisms include acid clearance; a mucosa specialised to withstand contact with acid; and the neutralising action of the bicarbonate-containing saliva that passes through the oesophagus ([Helm et al. 1982](#_ENREF_54)). The laryngeal mucosa lacks several of these protective mechanisms. There is no peristalsis, and the bicarbonate-rich saliva does not normally come into contact with the larynx. Intrinsic secretion of intra-cellular bicarbonate ions, a reaction catalysed by the family of carbonic anhydrase isoenzymes (CAs), takes place in both the oesophagus and the laryngo-pharynx ([Axford et al. 2001](#_ENREF_6); [Johnston et al. 2003b](#_ENREF_66); [Johnston et al. 2004](#_ENREF_68)). Eleven catalytically active forms of CAs are known, and have been found in several tissues in the gastrointestinal tract, such as the salivary glands, the laryngeal mucosa, the oesophagus, the gastric mucosa, the ileum, the jejunum and the colon ([Shousha and Pestonjee 1987](#_ENREF_140); [Parkkila and Parkkila 1996](#_ENREF_119)). The enzymes catalyse the reversible hydration of carbon dioxide, producing bicarbonate, which can neutralise refluxed gastric acid and increase the pH ([Helm et al. 1982](#_ENREF_54); [Tobey et al. 1989](#_ENREF_162)). Carbonic anhydrase isoenzymes I to IV are expressed in the oesophagus ([Christie et al. 1997](#_ENREF_23)), and in the larynx CA isoenzymes I to III are found (Axford et al., 2001). Patients with GORD show increased expression of CAIII in the inflamed squamous epithelium, which is presumably an attempt to mitigate damage ([Axford et al. 2001](#_ENREF_6)). In contrast, the laryngeal epithelial mucosa in patients with PL triggered by LPR lacks CAIII ([Johnston et al. 2003b](#_ENREF_66); [Johnston et al. 2004](#_ENREF_68)). Exposure of the larynx to pepsin and acid depletes the cells of CAIII and of the protective proteins such as squamous epithelial stress proteins (Sep53 and Sep70), an action not seen in the mucosa of the oesophagus ([Johnston et al. 2004](#_ENREF_68); [Johnston et al. 2006](#_ENREF_67); [Johnston et al. 2007](#_ENREF_69)). Pepsin has maximum activity at pH 2.0 and is inactive at pH 6.5 and higher. It remains stable until pH 8.0 ([Johnston et al. 2003a](#_ENREF_65); [Johnston et al. 2004](#_ENREF_68)). The mean pH of the laryngo-pharynx is pH 6.8. This can result in a reactivation of pepsin by a subsequent decrease in pH, which could occur during an episode of reflux or while eating and drinking food and beverages with a low pH ([Johnston et al., 2007a](#_ENREF_56)). Together, these findings may explain some of the similarities and differences in the pathophysiology of LPR and GORD. In addition, the patients who do not develop oesophagitis but PL may have been exposed to reflux where the refluxed fluid might have a different composition, or the mucosa of the larynx might be more sensitive to the refluxed contents.

### Different methods for measuring reflux

There are different ways of documenting reflux. The golden standard is the use of 24-h pH monitoring with dual probes; one probe placed distal to the upper oesophageal sphincter and one proximal to the LOS ([Smit et al. 2000](#_ENREF_146); [Fox 2011](#_ENREF_41)). However, 24-h pH monitoring with dual probes will register only acidic reflux. With combined, multi-channel intra-luminal impedance and pH testing, a technique based on the detection of changes in resistance to electrical currents, all types of reflux, acid reflux as well as non-acid and gaseous reflux, can be measured ([Tamhankar et al. 2004](#_ENREF_158); [Fox 2011](#_ENREF_41)). Impedance does not work well in the pharynx, and the patient often does not tolerate the catheter for dual probe 24-h pH monitoring, which makes it difficult to objectively measure reflux in patients with LPR. Recently, a minimally invasive device for detection of oral-pharyngeal reflux has been developed. The catheter can detect aerosolised acid and liquid and is better tolerated by patients than the catheter for dual probe measurements ([Sun et al. 2009](#_ENREF_154)). There are also commercially available tests for detecting the enzyme pepsin in saliva. As pepsin is produced only in the stomach, its presence in expectorated saliva indicates LPR ([Fox 2011](#_ENREF_41)). Ambulatory oesophageal pH monitoring using a wireless pH capsule system has become another common method for verifying reflux. The wireless pH capsule system has the advantage of extending the monitoring period up to 48 h ([Karamanolis et al. 2012](#_ENREF_74)). The capsule is placed proximal of the LOS and when measurements are finished it falls off, and is discarded with the patient´s normal bowel movements.

### Treatment for LPR-induced PL and GORD

Treatment recommendations are very similar for LPR and GORD. Patients with LPR-induced PL are recommended dietary modifications, as are patients with GORD. Avoidance of food that may induce reflux (coffee, alcohol, fatty foods, peppermint); avoidance of acidic food which can evoke heartburn (citrus, carbonated drinks, spices); and adopting habits that reduce oesophageal acid exposure (weight-loss, elevating the head and upper body at night, cessation of smoking) are recommended for both ([Kahrilas et al. 2008](#_ENREF_73)).

The recommended treatment for LPR has been proton pump inhibitors (PPI) at a dose of 20 mg twice daily for at least 2–3 months, as a single therapy or in combination with alginate preparations ([Barry and Vaezi 2010](#_ENREF_7); [Pearson et al. 2011](#_ENREF_123)). As single therapy, alginate preparations have also been shown to be an effective treatment for LPR ([McGlashan et al. 2009](#_ENREF_101)).

The recommended effective pharmacological treatment for GORD is PPIs at a dosage of 20 mg or 40 mg once daily for 6–8 weeks ([van Pinxteren et al. 2006](#_ENREF_170)). A twice daily dosing, if the once daily regime is not sufficient to resolve the patients symptoms, has also been recommended ([Kahrilas et al. 2008](#_ENREF_73)). Alginate suspensions have been proven effective as a single therapy or in combination with PPIs, to resolve heartburn ([Manabe et al. 2012](#_ENREF_96); [Pouchain et al. 2012](#_ENREF_131)).

As studies have shown that high-dose PPI treatment and anti-reflux surgery are equal treatment options to prevent oesophagitis in patients with GORD ([Lundell et al. 2007](#_ENREF_92)), anti-reflux surgery is recommended only to those who have adverse effects from PPIs or who experience troublesome regurgitation despite medical treatments. Patients with LPR may also benefit from anti-reflux surgery ([Kahrilas et al. 2008](#_ENREF_73)).

## Functional and motility disorders of the gastrointestinal tract

### Functional disorders

Functional gastrointestinal disease (FGID) is a gastrointestinal disorder so far not explained by structural abnormalities, infection, metabolic changes or abnormal gastrointestinal motility when measured ([Seres et al. 2008](#_ENREF_139)). There are several different kinds of FGID: functional heartburn, functional dyspepsia and functional oesophageal disease ([Sifrim and Zerbib 2012](#_ENREF_141)), but the one most common is irritable bowel syndrome (IBS) ([Hungin et al. 2003](#_ENREF_61)). There is a comorbidity of affective disturbances such as depression and anxiety in patients who have FGID ([Hungin et al. 2003](#_ENREF_61)). Moreover, patients who have one functional disorder often have additional functional disorders and/or GORD ([Aaron et al. 2000](#_ENREF_1); [North et al. 2004](#_ENREF_108); [Spiller et al. 2007](#_ENREF_150)). Furthermore, patients with FGID are thought to have a heightened perception to normal visceral stimuli, called visceral hypersensitivity, partly accounting for their symptoms ([Richter et al. 1986](#_ENREF_133); [Silverman et al. 1997](#_ENREF_142); [Kahrilas et al. 2011](#_ENREF_72)). The aetiology of visceral hypersensitivity has been assumed to emanate from one, or a combination of the following ([Gregory et al. 2003](#_ENREF_45)):

- sensitisation of afferent gut nerves

- sensitisation of spinal cord afferents

- abnormal brain processing e.g. a non-noxious sensation is misinterpreted as noxious as an effect of emotional or cognitive influence

Once central sensitisation has occurred it can continue to produce the perception of pain even if the initiating stimulus is discontinued ([Sifrim and Zerbib 2012](#_ENREF_141)). A similar aetiology has been suggested in the case of PL ([Kotby et al. 2010](#_ENREF_80)).

#### Functional oesophageal disease

There are several indications of functional oesophageal disease ([Kahrilas et al. 2011](#_ENREF_72); [Sifrim and Zerbib 2012](#_ENREF_141)). The patients have typical symptoms of reflux and sometimes display non-cardiac chest pain, have normal oesophageal mucosa seen at upper endoscopy and normal oesophageal acid exposure, but still experience strong correlation between physiological acid reflux events and symptoms ([Sifrim and Zerbib 2012](#_ENREF_141)). When experimentally testing oesophageal balloon distention in patients with non-cardiac chest pain, the patients are more sensitive to smaller volumes of balloon distention than controls ([Richter et al. 1986](#_ENREF_133)). This suggests the possibility of visceral hypersensitivity in these patients, and consequently a poor response to acid-suppressive therapy ([Pearson et al. 2011](#_ENREF_123)).

#### Irritable bowel syndrome

The prevalence of IBS in the normal population is 10%–20%. It is characterised by chronic abdominal pain in combination with altered bowel habits and affects predominantly women ([Longstreth et al. 2006](#_ENREF_91)). The pathophysiology of the disease is uncertain, but a subgroup of patients develops symptoms of IBS after an episode of infectious gastroenteritis ([Dunlop et al. 2003](#_ENREF_33)). Hormonal, dietary, and psychosocial mechanisms have also been suggested as a cause of the disease ([Levy et al. 2001](#_ENREF_89); [Simren et al. 2001](#_ENREF_143)). Patients who have IBS display heightened negative responses to stressful situations ([Elsenbruch et al. 2010](#_ENREF_34)) and have an inferior capacity for coping with them compared to the normal population ([Seres et al. 2008](#_ENREF_139)). The ROME III criteria, which are based on the patient´s symptoms, form the basis for the diagnosis ([Longstreth et al. 2006](#_ENREF_91)).

The clinical expression of IBS may vary. Thus, it is a multifaceted condition with a wide range of treatments. There is evidence that antispasmodics, probiotics and fibre supplements improve symptoms in patients who have IBS ([Spiller et al. 2007](#_ENREF_150)). Pharmacological treatment with anti-depressants of either the tricyclic type or selective serotonin reuptake inhibitors, treatment with cognitive behavioural therapy or hypnotherapy, can improve bowel habits and other symptoms ([Spiller et al. 2007](#_ENREF_150)).

### Motility disorders

Gastrointestinal motility is dependent on coordination between the intrinsic and extrinsic nervous system, the interstitial cells of Cajal and the smooth muscle cells of the gastrointestinal tract. The aetiology of motility disorders, such as oesophageal dysmotility, gastroparesis, chronic intestinal pseudo-obstruction (CIPO) and enteric dysmotility (ED), is in most cases unknown, but auto-immunity, diabetes mellitus and inflammation have been suggested ([Stanghellini et al. 1987](#_ENREF_152); [Spechler and Castell 2001](#_ENREF_149); [Wingate et al. 2002](#_ENREF_183); [Park and Camilleri 2006](#_ENREF_118)).

#### Oesophageal dysmotility

The main symptoms of oesophageal dysfunction are chest pain, dysphagia and heartburn ([Kahrilas et al. 2011](#_ENREF_72)). Various systemic diseases influence the oesophageal muscle. Degeneration of neurons in the oesophageal wall can produce achalasia ([Ghoshal et al. 2012](#_ENREF_44)). Gastro-oesophageal reflux, eosinofilic oesophagitis and altered levels of intestinal hormones affect oesophageal motility, but in many cases the primary cause of dysmotility is unknown (Spechler and Castell, 2001).

The diagnosis is set by oesophageal manometry, and sometimes by barium swallowing study and an upper endoscopy ([Van Trappen 1990](#_ENREF_171)). Efforts based on manometric results have been made to classify different forms of oesophageal dysmotility. These groups are ([Spechler and Castell 2001](#_ENREF_149)):

* inadequate LOS relaxation e.g. achalasia
* uncoordinated contractions e.g. diffuse oesophageal spasm, GORD
* hyper contraction e.g. nutcracker oesophagus
* hypo contraction e.g. ineffective motility in progressive systemic sclerosis

Therapy is often aimed at the underlying disease but in some cases directed at the dysmotility itself, as in the case of dilatation of the LOS in achalasia. In the cases of other motility disorders, e.g. diffuse oesophageal spasm and nutcracker oesophagus a variety of medications are used. These drugs aim to relax the muscles in the oesophagus. In the case of hypo contraction of the oesophagus prokinetic medicines can be used ([Pennathur et al. 1994](#_ENREF_126)).

#### Gastroparesis

Gastroparesis is defined as prolonged gastric emptying without mechanical obstruction ([Camilleri et al. 2012](#_ENREF_17)). The aetiology is multifactorial but the main causes are considered to be diabetic, post-surgical and idiopathic ([Park and Camilleri 2006](#_ENREF_118)). Patients who have gastroparesis experience nausea, vomiting, bloating and postprandial fullness. The majority of the cases are women ([Parkman et al. 2010](#_ENREF_120)). A solid-phase gastric emptying scintigraphy confirms the diagnosis and is considered the golden standard of investigation ([Parkman et al. 2004](#_ENREF_121)). Therapy is primarily focused on the underlying cause and prokinetic agents such as metoclopramide, cisapride, and erythromycin or other motilides have been used ([Park and Camilleri 2006](#_ENREF_118)).

#### Chronic intestinal pseudo-obstruction and enteric dysmotility

Severe forms of IBS may be difficult to differentiate from motility disorders such as CIPO and ED. The diseases are characterised by abdominal pain, abdominal distension, weight loss, nausea, vomiting, constipation, heartburn and dysphagia ([Törnblom 2008](#_ENREF_165)).

CIPO is a rare condition with the prevalence in Sweden of 3–5 cases per 100 000 inhabitants ([Törnblom 2008](#_ENREF_165)). The diagnosis requires abnormal contractile activity of the small intestine in combination with episodic or chronic signs mimicking mechanical obstruction ([Stanghellini et al. 1987](#_ENREF_152)). ED closely resembles CIPO, but has a less severe symptomatology and no signs of obstruction ([Wingate et al. 2002](#_ENREF_183)).

## Motilin

Motilin is an intestinal hormone consisting of 22 amino acids, which is produced in enterochromaffin cells in the duodenum and jejunum ([Christofides et al. 1981](#_ENREF_24)).

Motilin receptors are G-protein-coupled receptors, found in the mucosal layer and to a greater extent, in the muscle layer and the myenteric plexus of the distal part of the oesophagus, the stomach, the small intestine, and the large intestine in humans ([Takeshita et al. 2006](#_ENREF_157)). Ingestion of fat and gastric acid in the duodenum stimulates, and pancreatic bicarbonate counteracts, secretion of the hormone into the bloodstream ([Mitznegg et al., 1976](#_ENREF_92)). In dogs and in humans, motilin is known to increase motility in the oesophagus and augment LOS pressure ([Lux et al. 1976](#_ENREF_93); [Meissner et al. 1976](#_ENREF_102)), and it regulates the phase III of the migrating motor complex (MMC) ([Tomita et al. 1997](#_ENREF_163)). The MMC is initiated by the cells of Cajal in the antrum of the stomach and in the duodenum, and progresses along the small intestine to the colon. Its main purpose is to keep the stomach and intestines clear of debris ([Husebye 1999](#_ENREF_62)). Pressure variations of the LOS are partly associated with the MMC as to prevent gastro-oesophageal reflux ([Tomita et al. 1997](#_ENREF_163)).

Patients who have GORD, have defective LOS pressure and oesophageal dysmotility, and have been found to display altered motilin levels (Perdikis et al., 1994). The motilin levels, LOS pressure and oesophageal dysmotility are normalised after anti-reflux surgery ([Gardenstätter 2000](#_ENREF_43)). The LOS plays a crucial role in the reflux barrier. Thus, it has been of interest to find drugs that target the LOS and increase its pressure. Macrolides (erythromycin), better known as substances used as antibiotics, have been shown to mimic the actions of motilin, hence the nickname motilides. Accordingly, erythromycin increases LOS pressure and increases oesophageal motility, both in healthy subjects as well as in patients with GORD, by increasing the amplitude, duration, velocity, and strength of peristalsis ([Pennathur et al. 1993](#_ENREF_125); [Pennathur et al. 1994](#_ENREF_126); [Koutsoumbi et al. 2000](#_ENREF_85); [Chrysos et al. 2001](#_ENREF_25)).

As PL by some is perceived as an extra-oesophageal manifestation of GORD ([Ylitalo et al. 2004](#_ENREF_188); [Pearson et al. 2011](#_ENREF_123)), which is known to display altered levels of motilin ([Perdikis et al., 1994](#_ENREF_109)), it was pertinent to this thesis to investigate whether PL patients had altered levels of motilin as well.

## Gonadotropin-releasing hormone

Gonadotropin-releasing hormone (GnRH) is a decapeptide synthesised in the hypothalamus and secreted in a pulsatile manner into the hypothalamo-hypophyseal portal circulation ([Hazum and Conn 1988](#_ENREF_52)). It binds to specific G-protein-coupled receptors localised in the plasma membrane of the anterior pituitary and provokes secretion of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) ([Hazum and Conn 1988](#_ENREF_52); [Naor 2009](#_ENREF_105)).

FSH stimulates spermatogenesis in men and ovarian follicle growth and maturation in women. LH stimulates testosterone secretion in men and ovulation and formation of the corpus luteum in women ([Hsueh and Jones 1981](#_ENREF_59); [Hazum and Conn 1988](#_ENREF_52)). The secretion of GnRH is in turn regulated through negative feedback from the gonadal steroid hormones oestrogen, progesterone, and testosterone ([Maeda et al. 2010](#_ENREF_94)). At low, pulsatile doses, GnRH has the ability to stimulate reproductive functions but at high, continuous doses it has the opposite effect, suppressing reproductive actions by desensitising or down-regulating the receptor ([Khanna et al. 1992](#_ENREF_78); [Maeda et al. 2010](#_ENREF_94)). This action has been beneficial in clinical practice in the treatment of, for example sex hormone-related gynaecological pathologies such as endometriosis or precocious puberty, or in the treatment of hormone-dependent cancer ([Conn and Crowley 1994](#_ENREF_27)). The desensitising action is also used in the setting of in vitro fertilisation ([Maheshwari et al. 2011](#_ENREF_95)).

The GnRH-receptor (GnRH-R) has been discovered in a multitude of locations such as the brain, spinal chord, liver, pancreas, spleen, kidney, skeletal muscle, placenta, endometrium, ovary, mammary gland, testis, prostate, lymphocytes, gastric parietal cells, and in several forms of cancer and cancer cell lines ([Huang et al. 2001](#_ENREF_60); [Naor 2009](#_ENREF_105)).

### The role of GnRH in the gastrointestinal tract

The mode of action of GnRH is not well understood. As the hormone secreted from the hypothalamus has a half-life of 2–4 min and is secreted into the hypophyseal portal circulation ([Conn and Crowley 1994](#_ENREF_27)), concentrations are very low in peripheral circulation. It thus seems unlikely that hypothalamic GnRH is able to affect peripheral sites, such as the gastrointestinal tract ([Hazum and Conn 1988](#_ENREF_52)).

The ability to express the GnRH-R and to produce GnRH is found in the rat gastrointestinal tract in the surface epithelium, the glandular epithelium, and in the myenteric plexus ([Ho et al. 1996](#_ENREF_56); [Huang et al. 2001](#_ENREF_60)). GnRH has been shown to inhibit gastric secretion in dogs, probably mediated via a vagal cholinergic pathway ([Soldani et al. 1982](#_ENREF_148)), and it is suggested that GnRH, through paracrine and autocrine pathways, might influence glucose metabolism and insulin secretion ([Wang et al. 2009](#_ENREF_174)). The GnRH-analogue leuprolide restores motor function in the gastrointestinal tract in ovariectomised rats ([Khanna et al. 1992](#_ENREF_78)), whereas LH antagonises myoelectric activity in the small bowel in rats ([Ducker et al. 1996](#_ENREF_32)).

In humans, GnRH and LH receptors have been found in the ENS ([Ohlsson et al. 2007](#_ENREF_113); [Hammar et al. 2012a](#_ENREF_49); [Hammar et al. 2012b](#_ENREF_50)). Women with IBS have more symptoms from their IBS during certain parts of their menstrual cycles ([Heitkemper et al. 2003](#_ENREF_53)). This is thought to be due to the hormone progesterone, which could act as an antagonist on the ENS and reduce motor activity of the gastrointestinal tract ([Mathias and Clench 1998](#_ENREF_97); [Wang et al. 2003](#_ENREF_173)). When treated with the GnRH-analogues leuprolide or leuprolide-acetate, the symptoms are considerably reduced ([Mathias et al. 1994a](#_ENREF_98); [Mathias et al. 1994b](#_ENREF_99); [Palomba et al. 2005](#_ENREF_116)). The hypothesis behind its positive effects on IBS is that continuous treatment with leuprolide down-regulates the release of LH and FSH ([Maeda et al. 2010](#_ENREF_94)), and thereby are the hormones, which impair gastrointestinal motility, down-regulated ([Mathias et al. 1998](#_ENREF_100); [Wang et al. 2003](#_ENREF_173); [Maeda et al. 2010](#_ENREF_94)).

Antibodies against GnRH have recently been discovered in patients with IBS, CIPO, and ED, independently of GnRH treatment ([Ohlsson et al. 2010](#_ENREF_109); [Ohlsson et al. 2011](#_ENREF_112)). In some patients, the number of GnRH-containing neurons of the ENS was diminished compared to controls along with serum antibodies against GnRH ([Ohlsson et al. 2007](#_ENREF_113); [Hammar et al. 2012a](#_ENREF_49)).

As antibodies against GnRH have been found in patients with functional diseases and gastrointestinal dysmotility ([Ohlsson et al. 2011](#_ENREF_112)), it was relevant to this thesis to investigate whether patients with PL, which in part may be due to oesophageal dysmotility, is more prevalent in women, and where a functional aetiology has been suggested ([Kotby et al. 2010](#_ENREF_80)), also display similar antibodies.

## Health-related quality of life

### Definition

The definition of health originates from 1948 when the World Health Organization declared health to be *“a state of complete physical, mental, and social well-being, and not merely the absence of disease or infirmity”* ([WHO 1948](#_ENREF_180)). This has led to the following definition of health-related quality of life (HRQOL) used in HRQOL research and in this thesis: *“In relation to medical care and treatment, HRQOL is defined as a multidimensional concept covering all aspects of an individual's well-being, including their physical and mental health”* ([Fayers 2007](#_ENREF_37)).

### Why measure HRQOL?

Disease is not only diagnosed with the help of signs, but is perceived by the patient in the form of symptoms, thus HRQOL measures are important to convey the patient´s experience of the current medical care and treatment ([Wilson and Cleary 1995](#_ENREF_182)). HRQOL outcomes can be used in clinical trials of curative or palliative treatment, to measure the alleviation of symptoms, predict treatment success, register problems of psychosocial adaption after treatment, and provide information for policy decisions ([Guyatt et al. 1993](#_ENREF_48); [Ware 1995](#_ENREF_176); [Fayers 2007](#_ENREF_37)). Furthermore, measures of quality of treatment are often required by e.g. regional ethics review boards, health authorities and others.

The notion to measure HRQOL arose when we noted that patients were often only partially, or not at all, improved by medication for LPR-induced PL. In addition, we noted that studies made on HRQOL in this group of patients all pointed to a negatively influenced HRQOL ([Cheung et al. 2009](#_ENREF_22)). As previous studies indicated low HRQOL, and it seemed as if the conventional investigational procedure, care, and treatment of these patients was inadequate, it was important to register HRQOL in order to get an objective measure of the patients´ experience in order to try to ameliorate investigational procedures, and if needed, alter the treatment and care for this group of patients.

### HRQOL questionnaires

There are generic questionnaires, which can be used irrespective of the illness or condition measured. They provide a general summary of patient-reported HRQOL and may also often be applicable to healthy individuals (Fayers, 2007). Sometimes the HRQOL instruments need to focus on issues particular to a specific patient, a specific disease or a specific area of function ([Guyatt et al. 1993](#_ENREF_48)).

### HRQOL in posterior laryngitis

When we initiated our work in 2006, few studies had been undertaken in the area concerning the HRQOL of patients who have PL, and there was a lack of a validated and refined tool for measuring HRQOL within this entity. The studies previously done utilised The Medical Outcomes Study 36-Item Short Form (SF-36) in some cases, but also registration of HRQOL using the Voice Handicap Index, the domain-specific tool Hospital Anxiety Depression Scale and well-being in general by using the VAS ([Powitzky et al. 2003](#_ENREF_132); [Carrau et al. 2004](#_ENREF_19); [Siupsinskiene et al. 2007](#_ENREF_144)). In 2010, Andersson et al. ([Andersson et al. 2010](#_ENREF_3)) validated a Swedish translation of a combined tool, the LPR-HRQL, intended for HRQOL measurements in patients with LPR. By then we were well immersed in the studies forming this thesis and decided not to change the HRQOL tools we had started with.

The SF-36 questionnaire was originally constructed to gain an overview of the health status in the Medical Outcomes Study ([Ware and Sherbourne 1992](#_ENREF_177)). Initially, it was designed to be used in general population surveys, health policy evaluations, as well as in research and in clinical practice. It evaluates the perception of generic health, not specific to age, disease or treatment group, and stresses physical, emotional, and social functioning ([Ware and Sherbourne 1992](#_ENREF_177)).

The decision to use the SF-36 in this thesis was in part based on the aforementioned studies within the area that had utilised the same tool, making it possible for us to compare our up-coming results, but also because it is one of the most commonly used HRQOL instruments in the area of gastroenterology, an area tangent to our field of research ([Yacavone et al. 2001](#_ENREF_185)). In addition, the SF-36 is one of the most commonly used and most well-validated, general health-status measures in the world. There are representative studies facilitating comparisons within, and between, several diseases ([Ware 1996](#_ENREF_175)). The SF-36 questionnaire is divided into eight subscales of general health, arranged according to the degree to which they measure physical vs. mental health. Two additional dimensions can be calculated, physical (PCS) and emotional health (MCS), based on the weighting of the importance of the other eight subscales.

All of these factors, in total, made the SF-36 the HRQOL instrument of choice for our studies.

### Studies on HRQOL in posterior laryngitis

In 2003, a prospective study designed to characterise patients with typical GORD symptoms (e.g. regurgitations, heartburn) and extra-oesophageal symptoms of reflux was undertaken. To register HRQOL, SF-36 was used. The study established that the HRQOL did not differ between the group of patients who had LPR-induced PL or GORD, but compared to asymptomatic controls, the LPRgroup had significantly lower HRQOL in the majority of the SF-36 domains ([Powitzky et al. 2003](#_ENREF_132)).

In another study, SF-36 results were compared between patients with GORD and patients with PL but also to the general U.S. population. This study could detect that mean scores for patients with PL were significantly lower than those for patients with GORD in the domains SF and VT, but the HRQOL of the patients who had PL was also significantly worse in seven out of eight SF-36 domains compared to the general U.S. population ([Carrau et al. 2004](#_ENREF_19)).

Furthermore, in 2007, the HRQOL of patients with PL was compared to healthy controls. QOL was measured using the disease-specific questionnaire Voice Handicap Index, well-being in general, and the domain-specific tool Hospital Anxiety Depression Scale ([Siupsinskiene et al. 2007](#_ENREF_144)). The study indicated that patients who had PL had higher levels of anxiety, lower scores concerning well-being and social activity, and higher scores measured by the Voice Handicap Index, than the controls. The symptoms of PL had a significant relation to all measured QOL parameters, and all the QOL parameters improved significantly after three months of treatment with PPIs ([Siupsinskiene et al., 2007](#_ENREF_140)).

There are a few more studies that have been conducted within this area of research, all of them indicating that patients experiencing PL have an affected and sometimes low HRQOL ([Lenderking et al. 2003](#_ENREF_87); [Cheung et al. 2009](#_ENREF_22)).

# Aims

The overall aim of this thesis was to examine symptoms, physical signs, different aetiologies, and HRQOL in patients with PL and/or oesophageal dysmotility. The aims of the individual studies were as follows:

###### Paper I

To investigate how a group of patients, diagnosed as having PL, were examined, treated, and followed, to determine whether follow-up is needed, whether the patients still had symptoms arising from their PL after treatment with PPIs, and to register their current HRQOL.

###### Paper II

To investigate how many of the patients diagnosed as having PL had acid reflux in the proximal part of the oesophagus, altered motilin levels, gastrointestinal symptoms associated with FGID, and to register the PL patients’ HRQOL before and after treatment.

###### Paper III

To scrutinise consecutive patients with PL for the presence of GnRH antibodies and to examine the association between antibodies and other measured parameters.

###### Paper IV

To examine whether blood levels of motilin are related to symptoms and signs of dysfunction in the oesophagus and stomach in a well-defined group of patients with diabetes mellitus.

# Subjects and Methods

## Subjects

Between 2003 and 2011, a total of 224 patients were included in the studies (see Table 1 for patient characteristics). In paper I, the patients were retrospectively identified in medical records by searching for the following ICD codes: GORD with/without oesophagitis (K210, K219), chronic laryngitis (J370), laryngeal spasm (J385), other diseases of the vocal cords (J383), pain in the throat (R070), dysphagia (R139), and dysphonia (R490). The patients were invited to participate by letter. In paper II and IV, the patients were included gradually in a prospective manner. In paper II, 21 healthy volunteers served as controls for motilin levels and 83 volunteers served as controls for the VAS-IBS questionnaire. In paper III, subjects were a subpopulation from paper II, and for each patient, two age- and gender matched controls were randomly extracted from a cohort of healthy blood donors.

Table 1. Characteristics of patients and controls in the four papers

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Number of  controls | Number of  patients | Gender  (female/male) | Age range  (years) |
| Paper I |  | 122 | 76/46 |  |
| Paper II |  | 46 | 26/20 | 41-68 |
|  | 21 (m) |  | 14/17 | 36-55 |
|  | 83 (v) |  | 65/18 | 35-44 |
| Paper III |  | 37 | 20/17 | 40-69 |
|  | 74 (ab) |  |  |  |
| Paper IV |  | 19 | 10/9 |  |

m = motilin, v = VAS-IBS, ab = antibodies against GnRH.

The inclusion criterion was PL in all patients in paper I and II. The patients displayed typical signs by fibre laryngoscopy, in combination with one or several of the following symptoms, chronic cough, hoarseness, a sensation of having a lump in the throat (globus), excessive throat clearing, excessive phlegm, voice fatigue, throat pain and dysphagia ([Koufman et al. 2000](#_ENREF_82); [Hopkins C 2006](#_ENREF_58); [Pearson et al. 2011](#_ENREF_123); [Watson 2011](#_ENREF_178)). The inclusion criteria in paper IV were diabetes mellitus and the presence of simultaneous gastrointestinal symptoms.

In paper I, four patients were excluded, as they did not have the signs and symptoms of PL documented in the medical records. In papers II and III, 46 and 37, respectively, out of 60 invited patients with verified PL accepted to participate and were included in the studies. In paper IV, out of the 20 patients primarily included in the study, blood samples from 19 were further analysed to examine motilin concentrations in relation to oesophageal-gastric dysmotility.

## Methods

All studies were performed according to the Helsinki declaration, and were approved by the Regional Ethics Review Board at Lund University (approval numbers 145-02, 185/2009, 71/2007, 91/2008, 585/2012, 158/2007). Informed written consent was obtained from all the participants.

### Study design

Several methods were used in the different papers. An overview for each study is presented in table 2.

Table 2. Summary of the major methods used in this thesis

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Paper Ia  (N=122) | Paper II  (N=46) | Paper III  (N=37) | Paper IV  (N=19) |
|  | n (%) | n (%) | n (%) | n (%) |
| OGD | 49 (40) | 42 (91) | 37 (100) |  |
| 24-hpH monitoring | 25 (20) | 35 (76) | 32 (86) |  |
| Barium swallowing study | 41 (34) |  |  |  |
| Oesophageal manometry |  |  |  | 13 (68) |
| Gastric emptying scintigraphy |  |  |  | 19 (100) |
| Deep-breathing test |  |  |  | 17 (89) |
| Motilin |  | 37 (80) |  | 19 (100) |
| GnRH antibodies |  |  | 37 (100) |  |
| SF-36 I | 122 (100) | 44 (96) |  |  |
| SF-36 II |  | 40 (87) |  |  |
| VAS-IBS |  | 40 (87) |  |  |

n (%) = number and percentages of patients who have performed examinations. a In paper I, some patients went through more than one examination.

### Questionnaires

#### The 36-item Short-Form questionnaire (papers I, II)

SF-36 is an extensively used HRQOL instrument, which provides reproducible, reliable data on large populations, and has been shown to be useful as a global health monitor in clinical practice ([Winstead and Barnett 1998](#_ENREF_184)). It is available in Swedish, and Swedish reference data are available for many different conditions ([Sullivan M 2002](#_ENREF_153)). The SF-36 questionnaire is divided into eight subscales of general health, arranged according to the degree to which they measure physical vs. mental health. These subscales are physical functioning (PF), role functioning-physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role functioning-emotional (RE), and mental health (MH). Two additional dimensions can be calculated, physical (PCS) and emotional health (MCS), based on the weighting of the importance of the other eight subscales. The raw data were recoded at the time of analysis; the maximum score is 100, the higher the score the better the HRQOL.

#### The mGIS questionnaire (paper I)

Gastro-oesophageal reflux disease impact scale (GIS) is an instrument used to aid patient–physician communication concerning the symptoms of GORD and its treatment in a primary care setting (Jones et al., 2007). The mGIS questionnaire, which is based on the GIS questionnaire, contains modified questions more suited to symptoms experienced by patients with PL than GORD ([Pendleton et al. 2013](#_ENREF_124)). Background data (age, gender, tobacco and alcohol habits, voice demanding profession), current symptoms and anti-reflux medication were noted. It was also ascertained whether any of the patients had had reflux-associated symptoms during the previous 2 weeks despite medication, whether any of the patients considered it possible to manage without medication, and how often patients increased their medication. The last nine questions are designed to determine how often during the previous 2 weeks patients had experienced specific symptoms of posterior laryngitis, and how these symptoms had affected their daily life. The possible responses to the questions are: ‘‘always’’, giving a score of 1; ‘‘sometimes’’, a score of 2; ‘‘seldom’’ a score of 3; and ‘‘never’’, a score of 4. Thus, a high score indicates that the patient has few symptoms and a low score indicates more symptoms.

The Visual Analogue Scale for Irritable Bowel Disease (paper II**)**

Self-estimation of gastrointestinal symptoms was performed using the VAS-IBS questionnaire and was filled in at home at the time of the follow-up. It was previously developed and validated for patients with gastrointestinal symptoms without secured organic causes ([Bengtsson et al. 2007](#_ENREF_10)). Patients estimated seven different aspects of their condition on a visual analogue scale (VAS) from 0–100 mm, where 0 represents very severe problems and 100 represents absence of problems. These seven aspects are abdominal pain, diarrhoea, constipation, bloating and flatulence, vomiting and nausea, perception of psychological well-being and the intestinal symptoms´ influence on daily life. It also contains two additional questions concerning the patient´s sense of urgency to defecate and the feeling of incomplete evacuation after defecation. These questions were to be answered by “yes” or “no”.

### Ambulatory 24-h pH monitoring (papers II, III)

Ambulatory pH monitoring for proximal oesophageal reflux was performed for 24 h using an antimony pH electrode with an internal reference electrode (Versaflex, Sierra Scientific Instruments, Los Angeles, Ca, USA). The catheter was introduced through the nose and under fluoroscopic control positioned 5 cm below the UES (Philips Multidiagnost Eleva, CA, USA).

An episode of acid reflux was defined as a decrease in oesophageal pH to below 4 for more than 10 s. Previously established upper limits of normal acid exposure in clinical studies, with pH < 4 for 1% of total time, were used in the analysis of the data ([Dobhan and Castell 1993](#_ENREF_31); [Postma 2000](#_ENREF_128)).

### Measurements of motilin (papers II, IV)

Blood samples were taken into heparinised tubes. The plasma was separated and frozen at -20° C within 1 h of collection. Plasma motilin levels were measured with the help of a radioimmunoassay (RIA), using a rabbit antiserum (R-8423) raised against highly purified porcine motilin. The antiserum is directed to the NH2-terminal of motilin (amino acids 1–9). The coefficient of variation, intra-assay CV, is < 8% for controls at 140 pmol/l ([Sjölund et al. 1996](#_ENREF_145)).

### Measurement of human antibodies against GnRH (paper III)

Analysis of anti-GnRH antibodies was carried out by an enzyme-linked immunosorbent assay (ELISA) slightly modified on the basis of the results described in previous studies ([Ohlsson et al. 2009](#_ENREF_111); [Ohlsson et al. 2010](#_ENREF_109)). The wells of microtiter plates were coated with human GnRH and thereafter blocked with 0.5% fish gel solution (G7765, Sigma, St Louis, MO, USA) in phosphate-buffered saline (PBS) containing 0.05% Tween-20 (PBS-T). Serial dilutions of patient serum (1/100, 1/500 and 1/2500 in PBS-T) were then added to the plates. Deposition of auto-antibodies directed to GnRH was detected using biotinylated rabbit antihuman IgM or IgG antibodies. The bound, biotinylated antibodies were detected by alkaline phosphatase-conjugated streptavidin. To develop a colour reaction, a phosphatase substrate kit was used. The absorbance at 405 nm was measured after 2 h of incubation at room temperature. A plasma pool from healthy blood donors was included on each ELISA-plate for measurements of the variation. The plasma pool was used for the calculation of the intra-assay and inter-assay coefficients of variation, which for IgM were 11.5% and 16.1%, respectively, and for IgG 11.5% and 25.4%, respectively. Antibody levels are presented as relative units (RU) (absorbance values after subtraction of background levels and multiplied by 100). Relative units over 0 were considered as a positive antibody level ([Ohlsson et al. 2011](#_ENREF_112)).

### Oesophageal manometry (paper IV)

Standardised oesophageal manometry was performed with an intra-luminal, solid-state transducer system (Gaeltec Ltd, Ise of Skye, Scotland). All pressure values were expressed in mm Hg and referred to atmospheric pressure. The manometry catheter was introduced through the nose and fluoroscopically positioned in the distal oesophagus with the patient sitting in an upright position. All participants were instructed to swallow 10 ml of a barium contrast medium (60% w/v). At least five barium swallows were recorded. Patients who fulfilled one or more pathological value in the oesophageal manometry of the following five criteria were considered to suffer from oesophageal dysmotility ([Spechler and Castell 2001](#_ENREF_149)):

* absence of peristaltic contractions in the oesophagus (aperistalsis)
* mean peristaltic contraction amplitude < 30 or > 200 mmHg in the oesophagus
* more than 10% of the peristaltic waves in the oesophagus are simultaneous and non-propulsive
* speed of the peristaltic wave is < 3 or > 6 cm/s in the distal oesophagus
* resting pressure in the LOS of < 10 or > 30 mmHg

Normal peristaltic activity was defined as propulsive contraction waves with peak amplitudes between 30 and 200 mmHg and a speed between 3 and 6 cm/s.

### Gastric emptying scintigraphy (paper IV)

Gastric emptying scintigraphy was performed with the subjects in a semi-recumbent position. A test meal was prepared by adding 30–50 Mbq of technetium-99-labelled tin colloid to an egg, which was whipped in a glass cup while being heated in a water bath until coagulated. This was served with a piece of bread. The scintigraphy half time (T50) was identified from the point at which this trend line crossed the 50% value. Measurements of the radionuclide were corrected for decay according to Collins et al. ([Collins et al. 1983](#_ENREF_26)). T50 >2 standard deviations (SD) of healthy controls (=70 min) was considered abnormal ([Hanson and Lilja 1987](#_ENREF_51)).

### Deep-breathing test (paper IV)

The expiratory/inspiratory (E/I) ratio was calculated from the mean value of the longest R–R interval (on electrocardiogram) during expiration and the shortest R–R interval during inspiration. This is an established test of vagal, parasympathetic nerve function ([Sundkvist et al. 1979](#_ENREF_155)). All test results were expressed in age-related values and an age-related value below −1.64 SD was considered abnormal ([Forsen et al. 2004](#_ENREF_40)).

### Experimental procedure for meal-related plasma collection (paper IV)

All included subjects fasted overnight. The following morning they were given a fat-rich meal composed of 150 g cream and 150 g water without any seasoning. This generated 60 g fat and 561 kcal. This meal corresponds to the same content of fat as earlier used to provoke cholecystokinin (CCK) and oxytocin secretion ([Ohlsson et al. 2002](#_ENREF_110)). Blood samples were taken through an intravenous catheter 10 min and immediately before the injection, as well as 10, 20, 30, 45, 60, 90, 120, 150 and 180 min after the injection.

### Glucose monitoring (paper IV)

Using a glucose monitoring system (Mini Med, Sylmar, CA, USA), subcutaneous glucose levels were monitored continuously for 72 h ([Caplin et al. 2003](#_ENREF_18)).

## Statistical analyses

Statistical analyses were performed with SPSS 19-20 (Statistical Package for the Social Sciences). Parameters affected by age have been standardised using a linear regression model in which age was added as covariate. Parameters were then expressed as z-scores (paper II). For cases in which doubt arose regarding skewness, variables were analysed for normal distribution by the Kolmogorov-Smirnov test. The one-sample t-test was used to compare the data from the SF-36 questionnaire with the Swedish reference values (papers I and II). The Kruskal–Wallis test was used to compare numerical, independent data in the case of more than two groups (paper I) and the Mann-Whitney U-test when two groups were to be compared (papers II, III and IV). Wilcoxon´s signed test was used for comparisons between levels within the group (paper IV). Fisher´s exact test has been used for categorical variables (papers I, II, III and IV). Logistic regression analysis, adjusted for confounding factors, was used to calculate odds ratios with 95% confidence intervals (OR with 95% CI) for dichotomy variables (paper II). Correlations were calculated by the Spearman rank correlation test (papers II, III and IV). Results have been presented as median (interquartile range (IQR)) unless otherwise stated. P-values ≤ 0.050 were considered statistically significant.

# Results

## Symptoms and clinical managing of patients (papers I+II+IV)

The most common symptoms reported in paper I were hoarseness, followed by globus and coughing. A barium swallowing study in combination with OGD was the most common examination performed. Although reflux was the main suspected cause of the patients´ complaints, as many as 41% of the women and 37% of the men were not investigated for the presence of reflux. Despite this, 85% of these received acid-suppressing therapy with PPIs. The most common diagnosis set was GORD without oesophagitis (55%) and the second most common was chronic laryngitis (18%). Of the patients who had undergone investigational procedures, 90% of the women and 92% of the men were treated with PPI. Twenty-five percent of the women and 20 % of the men had no follow-up recorded in their journals.

The most common symptoms for the prospectively recruited patients in paper II were globus, excessive phlegm and hoarseness. The most common treatment prescribed was PPIs alone or in combination with alginic acid and 94% of the patients received acid-reducing therapy. All but two patients were followed up either by an appointment or by a consultation by telephone. The number of patients in paper I and II with various symptoms are displayed in table 3.

Table 3. The prevalence of the various symptoms in patients with posterior laryngitis

|  |  |  |
| --- | --- | --- |
|  | Paper I  (N= 122) | Paper II  (N=46) |
|  | n (%) | n (%) |
|  |  |  |
| Hoarseness | 47 (39) | 17 (37) |
| Globus | 42 (34) | 28 (61) |
| Coughing | 37 (30) | 11 (24) |
| Reflux | 27 (22) | 22 (48) |
| Excessive phlegm | 22 (18) | 22 (48) |
| Heartburn | 22 (18) | 12 (26) |
| Excessive throat clearing | 13 (11) | 11 (24) |
| Voice fatigue | 8 (7) | 12 (26) |
| Breathing difficulties | 19 (16) | 1 (2) |
| Feeling of cramp in the throat | 8 (7) | 0 (0) |

n (%) = number and percentage of patients with symptoms. More than one symptom for each patient was registered.

In patients with diabetes mellitus, abdominal fullness and gastro-oesophageal reflux (e.g. heartburn and acid reflux) were the only symptoms, which were significantly associated with delayed gastric emptying (paper IV). T50 was significantly longer in those patients who had abdominal fullness and gastro-oesophageal reflux than those without these symptoms. No symptom was associated with oesophageal dysmotility.

## Reflux (paper II)

Thirty-four percent, 12 of the 35 patients examined by 24-h pH monitoring, had a pathological reflux to the proximal oesophagus. The majority underwent OGD, and the most common findings were oesophagitis (n = 7), Barrett´s oesophagus (n = 10) and hiatal hernia (n = 14). Voice problems were associated with a pathological result from the 24-h pH monitoring (p = 0.050). Of the 12 patients complaining of voice problems, 11 were examined by OGD, and of these, five patients had a hiatal hernia, three had Barrett’s metaplasia, two had oesophagitis and one patient was positive for the bacterium *Helicobacter Pylori (H. Pylori)*. Two patients had normal OGDs.

## Antibodies against gonadotropin-releasing hormone (paper III)

The IgM antibody level was significantly higher in the patients with PL compared to controls (p = 0.007), and the prevalence of IgM antibodies against GnRH was in patients 35% compared to 28% in controls (p = 0.060). The level of IgG antibodies was the same in patients and controls (p = 0.700), but their prevalence of antibodies was 43% in patients and 4% in controls (p = 0.001). There was no association between the expression of IgM and IgG antibodies (p = 0.790), but one or both of these antibodies were found in 65% of the patients compared to 32% in controls (p = 0.002).

## Motilin (papers II+IV)

In patients who had PL, there were no differences in levels of motilin between patients and controls (p = 0.517) or between patients with a pathological 24-h pH monitoring result and those with a normal outcome (p = 0.057). However, the motilin levels differed between patients reporting reflux symptoms, i.e. heartburn and/or regurgitations (median 61(IQR 52–68) pmol/l), compared to those without reflux symptoms (median 71 (IQR 64–85) pmol/l) (p = 0.021) (paper II).

In paper IV, the time point for maximal motilin concentration after the test meal did not differ between patients with normal and those with an abnormal manometry result (p = 0.077). In patients with a pathological manometry result, a positive correlation between aperistalsis and percentage of simultaneous contractions in the manometry test was seen (rs = 0.699, p = 0.008). Also a correlation between the percentage of simultaneous contractions and basic motilin values (rs = 0.898, p = 0.006) and peak motilin values (rs = 0.842, p= 0.017) was seen. Basic concentrations of motilin correlated negatively to the mean peristalsis (rs = −0.900, p = 0.037). Patients who had gastro-oesophageal reflux had non-significant, higher levels of both basic and peak values of motilin compared to patients without reflux (89.2 (69.6–141.0) pmol/l and 63.5 (39.5–113.0) pmol/l, respectively, p = 0.098 and 152.5 (90.2–212.2) pmol/l and 83.0 (53.0–133.0) pmol/l, respectively, p = 0.063).

## Health-related quality of life (papers I+II)

### 36-item Short-Form questionnaire

#### At inclusion (paper II)

At the time of inclusion in the study, the women displayed significantly lower scores for all subscales, except for PF that did not differ from that for the Swedish female population (p = 0.264) or from the general Swedish population (p = 0.116). However, the men did not display any differences in scores compared to the general Swedish population, but the score for MH was significantly lower in the PL group compared to the Swedish male population (p = 0.050) (Figure 2).



**Figure 2**. Analysis of the SF-36 questionnaire at the time of inclusion of the population of men and women with posterior laryngitis and the general Swedish population. Gender- and age-matched values are presented as mean values. PF = physical functioning, RP = role-physical, BP = bodily pain, GH = general health, VT = vitality, SF = social functioning, RE = role-emotional, MH = mental health.

#### At follow-up (papers I+II)

At follow-up in paper I, the women had significantly lower scores (p < 0.001) for all subscales compared to the general and Swedish female population, except for the subscale PF that did not differ compared to the Swedish female population (p = 0.148). The women also had significantly lower scores for all subscales compared to the men participating in the study (Figure 3). In men, the only subscales to differ from those of the general Swedish population were PF (p = 0.015), which was higher and GH (p = 0.010), which was lower (Figure 3). No difference was noted compared to the general Swedish male population (data not shown).



Figure 3. Analysis of SF-36 at follow-up in paper I in the population of women and men with posterior laryngitis and the general Swedish population. Gender- and age-matched values are presented as mean values. PF = physical functioning, RP = role-physical, BP = bodily pain, GH = general health, VT = vitality, SF = social functioning, RE = role-emotional, MH = mental health.

In paper II at follow-up, the women had a significantly lower score for the subscale GH compared to the general Swedish population (p = 0.046) (Figure 4), but no significant difference was noted compared to the Swedish female population (data not shown). In men, no differences in scores were found compared to the general Swedish population (Figure 4) or to the Swedish male population (data not shown). The total PL population had significantly lower scores in the subscales RP (p = 0.047), GH (p = 0.016), and VT (p = 0.028) compared to the general Swedish population.



Figure 4. Analysis of the SF-36 questionnaire at follow-up in paper II in the population of men and women with posterior laryngitis and the general Swedish population. Gender- and age-matched values are presented as mean values. PF = physical functioning, RP = role-physical, BP = bodily pain, GH = general health, VT = vitality, SF = social functioning, RE = role-emotional, MH = mental health.

## Follow-up (papers I+II)

### mGIS questionnaire (paper I)

More women than men completed the mGIS questionnaire and the analysis showed that 29% (20/70) of the women had low scores (i.e. scores below 18). None of the men had scores below 18 (the highest attainable score is 36). The majority of patients, 63% (51 women, 26 men), still had symptoms at the time of completing the questionnaire and about 1/3 of them always or sometimes experienced sore throat/hoarseness, excessive throat clearing and globus, all of which are symptoms related to PL. In addition the symptoms experienced by the patients often prevented them from eating and drinking. About 1/3, reported that the symptoms of their PL affected their daily activities, thus having a negative impact on their lives.

### VAS-IBS questionnaire (paper II)

The analysis of the VAS-IBS questionnaire showed that women in the PL group registered significantly lower scores than the controls concerning abdominal pain (p = 0.001). Even the men in the PL group rated their symptoms as more severe than the controls, with a significant difference concerning abdominal pain

(p < 0.001), bloating and flatulence (BF) (p = 0.042), perception of psychological well-being (PW) (p = 0.024) and the intestinal symptoms´ influence on their daily life (p = 0.020) (Figure 5). There was no difference in VAS-IBS between those who expressed, or did not express, IgM or IgG antibodies against GnRH at inclusion (data not shown).

Figure 5. Analysis of the VAS-IBS questionnaire, expressed as median z-scores, for the PL group and the controls at follow-up. AP = abdominal pain, D = diarrhoea, C = constipation, BF = bloating and flatulence, VN = vomiting and nausea, PW = perception of psychological well-being, IDL = intestinal symptoms´ influence on daily life. Mann-Whitney U-test. P ≤ 0.050 was considered statistically significant. \* = Significant difference to controls-women, \*\* = Significant difference to controls-men.

# Discussion

In this thesis, we have shown that LPR as the aetiology of PL occurs to a lesser extent than expected, as only 1/3 of the patients displayed objectively measured reflux. The majority of the patients at an ENT clinic were being treated with PPIs, although many of them had not undergone any investigation to confirm LPR. Despite PPI treatment, the majority of patients had symptoms at follow-up, indicating refractoriness to PPIs. A high prevalence of functional gastrointestinal symptoms and antibodies against GnRH suggest a functional genesis in a subgroup of patients. Altered motilin levels in patients with reflux symptoms suggest an association between oesophageal dysmotility, LOS pressure and reflux in patients with PL.

Signs of PL have been observed in healthy subjects without any association to typical symptoms of PL or LPR ([Belafsky et al. 2001](#_ENREF_9)). In a study, which investigated the laryngeal signs and symptoms associated with LPR, it was found that LPR does not render specific laryngeal symptoms ([Ylitalo et al. 2001](#_ENREF_187)). This is in accordance with other studies, indicating that there is an incomplete degree of concordance between symptoms, findings and reflux in PL ([Ylitalo et al. 2001](#_ENREF_187); [Bove and Rosen 2006](#_ENREF_15)). In addition, LPR episodes are present in most healthy adults ([Andersson et al. 2006](#_ENREF_4); [Sun et al. 2009](#_ENREF_154)), and there is still no consensus on the number of reflux episodes to the pharynx that is to be considered abnormal. Moreover, Kotby et al. ([Kotby et al. 2010](#_ENREF_80)) have pointed out that the symptoms considered typical for LPR and PL, e.g. the symptom globus, are nonspecific and may have causes other than LPR.

At present, the terminology concerning PL and LPR is unclear and the terms are used as if they are synonymous. Patients included in studies are said to have signs and symptoms of LPR when they actually have PL and vice versa. In order to distinguish between the two terms, it is important to use the right terminology. LPR is a back flow of gastric contents into the laryngo-pharynx, and its presence can be established by objective measurements, while PL is defined by symptoms in combination with inflammation involving the most posterior part of the glottal region, seen using laryngoscopy. It can be caused by LPR, but may have several other aetiologies ([Bove and Rosen 2006](#_ENREF_15); [Kotby et al. 2010](#_ENREF_80); [Pearson et al. 2011](#_ENREF_123)).

Inflammation of the larynx is seen in patients both with and without symptoms of PL or LPR. Patients with symptoms, but without signs of inflammation, might have a sensitisation of afferent nerves after a previous infection, or a non-noxious sensation can be misinterpreted as noxious as an effect of emotional or cognitive influence, while patients with signs of inflammation, but who lack symptoms, might process afferent nerve input differently and are thus without symptoms.

The use of the two terms, as if they were equivalent, have led us to believe that PL is the same thing as LPR and made us focus solely on acidity as the cause of PL. Thus, patients with PL are treated with PPIs even though little improvement of their symptoms is noted after using acid-suppressing agents and when there is little evidence of acid reflux into the laryngo-pharynx. This is in accordance with a study performed by Ylitalo et al. ([Ylitalo et al. 2001](#_ENREF_187)), where only about 2/3 of the patients who had typical signs and symptoms of PL had proven pharyngeal reflux when measured by 24-h pH monitoring. Laryngeal-pharyngeal reflux is hard to register and an ideal method has not been established, making a correct diagnosis difficult ([Kotby et al. 2010](#_ENREF_80)). Although other aetiologies may be the underlying cause of PL, it is still treated as if it were caused mainly by extra-oesophageal reflux ([Hopkins C 2006](#_ENREF_58); [Pearson et al. 2011](#_ENREF_123)). Moreover, 25% of the women and 20% of the men in paper I appeared not to have been followed up, even though they had a prolonged history of symptoms, and were being treated with PPIs. This goes against the recommendations for the use of this expensive and widely prescribed medication ([Khan et al. 2006](#_ENREF_77); [Vaezi et al. 2006](#_ENREF_168)).

There are several explanations for our patients´ refractoriness to PPI treatment, apart from the fact that PL may be induced by aetiologies other than reflux. LPR was measured by 24-h pH monitoring, but this only registers acidic fluids. PPIs reduce the acidity of the gastric juice, and reduce the volume of the reflux, but it does not prevent reflux episodes, leaving the possibility for weakly acidic, gaseous or non-acid reflux as being other mechanisms behind PL induced by LPR ([Tamhankar et al. 2004](#_ENREF_158); [Tack 2005](#_ENREF_156)). Moreover, FGID is known to be associated with visceral hypersensitivity ([Richter et al. 1986](#_ENREF_133); [Silverman et al. 1997](#_ENREF_142)), which has been suspected of causing non-cardiac chest pain, refractory to PPI treatment. Patients with FGID are known to have a comorbidity of affective disturbances such as depression and anxiety ([Elsenbruch et al. 2010](#_ENREF_34)), which has also been noted in patients with PL ([Siupsinskiene et al. 2007](#_ENREF_144)). It has been noted that patients who experience psychological distress, e.g. depression, have a weak response to PPI treatment ([Nojkov et al. 2008](#_ENREF_107)). Our results are similar of those studies concerning functional oesophageal disease ([Kahrilas et al. 2011](#_ENREF_72)), and indicate that PL may in part be a functional disease in which the patients display visceral hypersensitivity in the laryngo-pharynx. These findings in combination could further explain the lack of efficacy of PPI.

The role of the bacteria *H. pylori* in GORD and PL/LPR is unclear but treatment of *H. pylori* significantly improves GORD symptoms ([Saad et al. 2012](#_ENREF_134)). In the airways, *H. Pylori* has been found in saliva, oral lesions, tonsils, the adenoids and in sino-nasal tissues ([Li et al. 1995](#_ENREF_90); [Unver et al. 2001](#_ENREF_166); [Morinaka et al. 2003](#_ENREF_103)). The combination of LPR and *H. Pylori* infection has been suggested as a cause of laryngeal pathologies ([Tezer et al. 2006](#_ENREF_159)). One study has shown a major presence of *H. pylori* in lesions of the vocal folds ([Tiba et al. 2010](#_ENREF_160)), while other studies have failed to establish a connection between infection with the bacteria in either the stomach or the larynx, and LPR ([Ylitalo 2006](#_ENREF_186); [Cekin et al. 2012](#_ENREF_20); [Saruc et al. 2012](#_ENREF_136)). Thus, it is obscure whether *H. pylori* can be an additional aetiology of PL or LPR.

Although PL renders symptoms and reduces HRQOL (paper I and II), it has not yet been proved to have any severe or harmful outcomes for the patient. Thus, it is important that the treatment does not hurt the patient. The use of PPI in the Swedish population is estimated to be 5%–10%. It is an expensive medication and the prescription of the drug is increasing steadily ([Socialstyrelsen 2012](#_ENREF_147)) (National Board of Health and Welfare). Apart from the economic aspect, increased risk of pneumonia, intestinal infections, osteoporosis and a reduced effect of prophylaxis with aspirin and clopidogrel in cardiovascular diseases have been reported as adverse reactions to long-term treatment with PPI ([Leonard et al. 2007](#_ENREF_88); [Charlot et al. 2011](#_ENREF_21); [Eom et al. 2011](#_ENREF_35); [Ngamruengphong et al. 2011](#_ENREF_106)). The reduced production of hydrochloric acid due to PPI treatment has been shown to reduce the bactericidal effects in the stomach, thereby increasing the risk of pneumonia and infectious bowel diseases ([Leonard et al. 2007](#_ENREF_88); [Eom et al. 2011](#_ENREF_35)). The higher pH level alters the absorption of calcium, iron, vitamin B12, magnesium, and drugs ([Ito and Jensen 2010](#_ENREF_64); [Charlot et al. 2011](#_ENREF_21)). The majority of patients who have PL in our studies were women, who more often face the risk of osteoporosis and often have low values of iron ([WHO 2003](#_ENREF_181); [Ito and Jensen 2010](#_ENREF_64)). Considering the recent reports on the adverse effects of PPI on osteoclasts and absorption of calcium, iron, and vitamin B12, one should be cautious with PPI treatment in this group of patients, especially when the diagnosis is uncertain ([Ngamruengphong et al. 2011](#_ENREF_106)).

It has been repeatedly noted that women have a higher prevalence of functional and motility disorders in the gastrointestinal tract compared to men ([Spiller et al. 2007](#_ENREF_150)). This may not be due to that the prevalence of the disease in women is actually higher, but may reflect the reality that women are known to process the sensation of pain and discomfort differently than men ([Lee et al. 2001](#_ENREF_86); [Ihlebaek et al. 2002](#_ENREF_63); [Naliboff et al. 2003](#_ENREF_104)), and that they contact the health care system more frequently than men ([Briscoe 1987](#_ENREF_16); [Bertakis et al. 2000](#_ENREF_12)). This may be the explanation for the female predominance also in our studies of PL. In this thesis, patients with PL had lower scores on the VAS-IBS, especially the men, which indicate more gastrointestinal symptoms compared to controls. Sadik et al. ([Sadik et al. 2008](#_ENREF_135)) found in a group of patients with severe, unexplained gastrointestinal symptoms that gastrointestinal transit abnormalities were more common in men. The VAS-IBS results in our study (paper II) are in agreement with these results (Sadik et al., 2008), and suggest that the prevalence of gastrointestinal disorders may not differ greatly between the genders. The knowledge that FGIDs often overlap, and that patients may have more than one such disorder ([Whitehead et al. 2002](#_ENREF_179); [North et al. 2004](#_ENREF_108)), suggests that our patients´ laryngo-pharyngeal complaints might also have a functional origin. In addition, patients with FGIDs display antibodies against GnRH ([Ohlsson et al. 2011](#_ENREF_112)), which further strengthens the likelihood of a functional component in our patients with PL, who also displayed such antibodies.

The role of GnRH in the gastrointestinal tract is still unclear, but antibodies against GnRH have been found in patients with functional diseases and gastrointestinal dysmotility ([Ohlsson et al. 2007](#_ENREF_113); [Ohlsson et al. 2010](#_ENREF_109); [Ohlsson et al. 2011](#_ENREF_112)), where some patients display a diminished number of GnRH-containing neurons of the ENS compared to controls ([Ohlsson et al. 2007](#_ENREF_113); [Hammar et al. 2012a](#_ENREF_49)). Auto-antibodies against peptides such as insulin and vasoactive intestinal peptide are present in healthy subjects ([Sebriakova and Little 1973](#_ENREF_138); [Paul et al. 1989](#_ENREF_122); [Fetissov et al. 2008a](#_ENREF_38); [Fetissov et al. 2008b](#_ENREF_39)). It has been suggested that auto-antibody formation is a physiological phenomenon as gut microbial antigens are involved in the production of auto-antibodies common to all humans, e.g. agglutinins characterized for the ABO blood groups ([Springer and Horton 1969](#_ENREF_151)). The physiological formation of antibodies is one explanation for the antibodies against GnRH found in patients with FGIDs, gastrointestinal motility disorders and PL (paper III) ([Ohlsson et al. 2010](#_ENREF_109); [Ohlsson et al. 2011](#_ENREF_112)). Furthermore, there is evidence that pathogenic microorganisms, such as bacteria, viruses and fungi, have protein sequence homologous with endogenously produced hormones ([Oldstone 1998](#_ENREF_114); [Oldstone 2005](#_ENREF_115); [Fetissov et al. 2008a](#_ENREF_38); [Fetissov et al. 2008b](#_ENREF_39)). If the patient is infected, the alien microorganisms can induce the formation of an antibody that fights the infection, but can also evoke the formation of auto-antibodies, due to the homologous protein sequences, which can react to endogenously produced hormones, e.g. GnRH. Another explanation for auto-antibody formation may be that the infection, or any other agent, can cause tissue damage, exposing GnRH in the GnRH-producing neurons to inflammatory cells, and the antibodies rather represent an epiphenomenon to tissue damage. The latter mechanism is the most plausible explanation for the GnRH antibodies seen in PL, gastrointestinal motility disorders and FGIDs, and due to the reduction of GnRH-containing neurons of the ENS, seen in patients with gastrointestinal motility disorders ([Ohlsson et al. 2007](#_ENREF_113); [Hammar et al. 2012a](#_ENREF_49)).

Patients with GORD may have oesophageal dysmotility, defective LOS pressure and abnormal motilin levels compared to healthy subjects ([Kahrilas et al. 1986](#_ENREF_71); [Gardenstätter 2000](#_ENREF_43)). The present study of motilin in patients with PL (paper II) could not reveal a difference in levels of motilin between patients and controls, but patients with typical reflux symptoms had abnormal levels of motilin compared to those without these symptoms, which is in concordance with earlier studies in GORD ([Perdikis et al. 1994](#_ENREF_127); [Gardenstätter 2000](#_ENREF_43)). Our results suggest that motilin might be of importance for the LOS function and oesophageal motility in PL as well.

Oesophageal dysmotility is also common in diabetes mellitus and has been found in 68% of the cases ([Gustafsson et al. 2011](#_ENREF_47)). One study has shown that patients with diabetes mellitus have fewer symptoms of dysphagia if they also, in addition to aperistalsis, have a high percentage of simultaneous contractions in the oesophagus ([Faraj et al. 2007](#_ENREF_36)). Among our patients with diabetes mellitus, we found that there was a significant correlation between the percentage of oesophageal simultaneous contractions, and basic and peak plasma motilin values (paper IV). Altered levels of motilin resulting in the presence of simultaneous contractions may be a way to compensate and improve dysmotility, making the swallowing process more effective in these patients. On the other hand, it might be secondary to worsened oesophageal function with subsequent altered secretion of several gastro-duodenal hormones, which affect oesophageal motility negatively ([Borg et al. 2009](#_ENREF_13)). The relationship between simultaneous contractions, and basic and peak plasma motilin values, might apply to patients with PL and LPR, who are known to have oesophageal dysmotility. Further studies are required in this area to verify this.

It is highly credible that there are subgroups within PL, where some patients may have reflux, some display dysmotility of the oesophagus, and some patients have a functional disease.

Studies previously performed measuring the HRQOL in patients with PL show affected and decreased HRQOL ([Lenderking et al. 2003](#_ENREF_87); [Siupsinskiene et al. 2007](#_ENREF_144)). Both studies registering HRQOL in this thesis show low HRQOL, especially for women compared to the general Swedish and the Swedish female population (papers I and II). The lower HRQOL of women might reflect the propensity of women to complain about their symptoms ([Dimenas et al. 1990](#_ENREF_30); [Tibblin et al. 1990](#_ENREF_161); [Simren et al. 2001](#_ENREF_143)). The improvement of HRQOL seen in paper II might be due to adequate treatment, as has been noted in an earlier study ([Siupsinskiene et al. 2007](#_ENREF_144)). Receiving a diagnosis, receiving information about the nature of their disease, or being taken care of might also be the cause of the amelioration seen. Bengtsson et al. ([Bengtsson et al. 2006](#_ENREF_11)) have shown that women with a functional disease who feel that they are being taken care of, and are receiving information/education, improve their quality of life. The improvement of HRQOL in our study might be similar in nature (paper II).

As HRQOL in PL is clearly reduced and the current treatment is insufficient, a change of care for this group of patients is called for. Patients who display signs and symptoms of PL should go through 24-h dual probe pH monitoring or 24-h combined multi-channel intra-luminal impedance and pH testing, in combination with an OGD. If the measurements and/or the OGD show signs of reflux the patients should receive information to avoid food that may induce reflux; avoid acidic food which can evoke heartburn; and adopt habits that reduce oesophageal acid exposure. They should be treated with PPIs in combination with alginate suspensions and be followed-up 8–12 weeks later. If improved, medication should be gradually reduced. If symptoms remain unchanged or are only partly improved, and other causes of PL, e.g. allergy, voice abuse, smoking and alcohol abuse, have been ruled out, dysmotility or functional disease should be considered. If oesophageal dysmotility is suspected, it should be confirmed by manometry, and prokinetic therapy might be of value to ameliorate symptoms and refine the treatment of these patients. In FGIDs, there is evidence that pharmacological treatment with anti-depressant drugs, tricyclic or selective serotonin reuptake inhibitors, or treatment with cognitive behavioural therapy and hypnotherapy, improve bowel habits and symptoms ([Spiller et al. 2007](#_ENREF_150)). These therapies should also be considered for the patients suspected of having a functional form of PL.

## Strengths and limitations

The strengths of this thesis are that the studies originate from clinical observations found in patients with PL, who did not respond to acid-suppressing drugs. This thesis has tried to examine and identify different aetiologies and explanations for the symptoms, other than acidic reflux. In addition, it encompasses both retrospective and prospective studies, including both small and large study populations of patients. The findings in this thesis that functional aspects and intestinal hormones might be involved in the pathogenesis of the entity PL, and thus that treatments other than PPI can/should be considered, must be regarded strengths as they are, to a high degree, new in this area of research.

The limitations of this thesis are several. In some of the studies the number of participants was small, but sometimes it is necessary to perform small pilot studies before testing in large cohorts of patients. In addition, the information from the medical journals in paper I was collected in a retrospective manner. Also our controls for motilin and VAS-IBS were entirely or partly recruited from hospital staff, who may be healthier than the average individual. The matching of control and study groups may have been better, and we have tried to compensate for this shortcoming.

In paper I, the response rate was 69%. Seventy-two percent of those who answered were willing to participate in the study. Although the response rate was fairly high, the risk of overrepresentation of patients with a more severe condition is evident, as it is likely that they would be motivated, to a higher degree, to participate than patients with less severe disease. This may have influenced our results.

Another flaw is the absence of objective examinations of oesophageal dysmotility in the PL patients, which would have been an improvement.

Finally, data concerning smoking and alcohol habits were missing from the medical records (papers I and II), and the patients’ use of alcohol and tobacco was also missing on the mGIS questionnaire (paper I). This information would have been important as smoking and alcohol can cause PL. Probably the ENT-specialist had not thought about smoking and alcohol abuse as the aetiology of PL, but had focused solely on acidity being the cause of the patients’ complaints. Concerning the patients, it is likely the patients wanted to avoid disclosing this kind of sensitive information.

## Future perspectives

The studies in this thesis hint that current treatment is not optimal for patients with PL. Concerning LPR, previous studies have shown that it might not be the hydrochloric acid in the stomach contents that harms the mucous membranes of the larynx. It seems that pepsin has a major role in causing damage ([Johnston et al. 2004](#_ENREF_68)). There are research findings implying that other components of the refluxed stomach contents might be noxious as well, e.g. bile acids or non-acid reflux ([Vaezi and Richter 1995](#_ENREF_167); [Galli et al. 2003](#_ENREF_42); [Adhami et al. 2004](#_ENREF_2); [Kawamura et al. 2004](#_ENREF_76)). Further studies, including long-time recording for bile reflux to the proximal oesophagus or the laryngo-pharynx, could be of interest to determine an additional cause of PL. Furthermore, discussions are still on-going about how many reflux episodes to the laryngo-pharynx should be considered pathological. Moreover, the prevalence of PL or LPR is not clear and the distinction between PL and LPR needs to be clarified. All these matters need to be solved to increase our knowledge of different aetiologies for PL.

When starting the treatment used for FGIDs in patients suspected of having functional PL, SF-36 and/or the mGIS could be used, to note whether the altered therapy improves HRQOL in this group of patients.

Currently, knowledge about the prevalence of oesophageal dysmotility in patients with PL has been only sparsely examined ([Knight et al. 2000](#_ENREF_79)). A study measuring basal and peak levels of motilin, MII-pH or 24-h pH monitoring with dual probes, and oesophageal manometry would be of interest to further determinate the role of dysmotility and motilin levels in patients with PL caused by LPR. In addition, studies to elucidate whether prokinetic drugs, e.g. motilides, are of use could be performed.

The knowledge about how GnRH and antibodies against GnRH affect the gastrointestinal tract is still only rudimentarily explored. As PL patients display antibodies against GnRH, it would be of interest to know the effect of how GnRH or antibodies against GnRH influence visceral sensitivity, oesophageal motility and the LOS.

# Conclusions

Patients with PL still have symptoms several years after diagnosis. They are treated with PPIs even though sparse improvement of their symptoms is noted after using acid-suppressing agents for several years and when only a minority have evidence of acid reflux into the proximal oesophagus. Other aetiologies, such as non-acid reflux, weakly gaseous reflux acid and visceral hypersensitivity or oesophageal dysmotility for their complaints, are not considered.

Posterior laryngitis, in a subgroup of patients, may be due to a functional disease, especially in men, as they have concomitant functional gastrointestinal symptoms and only 1/3 have proximal acid reflux. Furthermore, patients with PL express antibodies against GnRH in serum at higher prevalence, compared to controls, in the same manner as in FGIDs and motility disorders, suggesting that PL might be a functional disease.

Plasma motilin concentrations vary with abnormalities in oesophageal motility in patients with diabetes mellitus. Motilin is also affected among PL patients with typical reflux symptoms, who have lower values compared to patients without typical reflux symptoms.

HRQOL is low in patients with PL compared to the general Swedish population, especially in women, but improves over time.

Overall, PL is associated with acid reflux in the proximal oesophagus, endocrine disturbances, functional gastrointestinal symptoms and reduced HRQOL. To ameliorate follow-up and by trying to optimise investigations and treatment, HRQOL can be improved.

# Populärvetenskaplig sammanfattning

## Inflammation i struphuvudet kan bero på annat än reflux

Bakre laryngit är en form av kronisk inflammation som påverkar struphuvudets bakre del och ibland stämbanden samt ingången till matstrupen. Det kan ge upphov till symtom i form av hosta, heshet, ökad slembildning och klumpkänsla i halsen. Inflammationen kan orsakas av flera olika faktorer såsom infektion, rökning, alkohol, allergi och felaktigt röstbruk. Den faktor som man oftast antar orsaka bakre laryngit är reflux, d.v.s. att magsaft går i riktning från magen, upp till struphuvudet. Det finns en studie som pekar på att 50-55% av alla patienter som har heshet också besväras av denna typ av reflux. Man behandlar dem oftast med magsyrehämmande läkemedel som t ex Losec, omeprazol, esomeprazol etc men det är inte alla som blir hjälpta av detta. Människor som har reflux, liksom människor som har diabetes, har ofta dålig rörlighet i matstrupen och förändrat tryck i matstrupens nedre ringmuskel. Detta kan vara ytterligare en orsak till reflux.

Motilin är ett hormon som bildas i tunntarmen och som är viktigt för regleringen av motoriken av mage och tarm. Receptorn för motilin finns i hela magtarmkanalen, även i matstrupens nedre del, och effekten utövas direkt på både nerv och muskel. Flera olika läkemedel som har samma effekt som motilin, har utvecklats för behandling av reflux och vid långsam magsäckstömning.

Gonadotropin-releasing hormon (GnRH) är mest känt för att styra utsöndringen av könshormonerna från hypofysen. Nyligen har man funnit att dessa hormon och dess receptorer också finns i magtarmkanalen och att de har betydelse för en normal magtarmfunktion. Patienter kan ha funktionella tarmbesvär, d.v.s. de har buksmärtor som förbättras av tarmtömning, ändrad tarmtömningsfrekvens och/eller avföringskonsistens utan att man kan hitta några bevis för sjukdom i tarmen. Det är dessutom känt att de som har funktionella tarmbesvär även ofta har huvudvärk, muskelvärk, depression mm. De har en sänkt smärttröskel i magen, s.k. visceral hypersensitivitet, och antikroppar mot GnRH i blodet. Forskning visar att patienter med funktionella tarmsjukdomar kan förbättras om de behandlas med probiotika, läkemedel som motverkar kramper i tarmen och/eller olika läkemedel mot depression. Hypnos och kognitiv beteendeterapi har också visat sig ha effekt.

Upprinnelsen till denna avhandling är observationen att patienter hos vilka man misstänker reflux, som orsak till inflammation i struphuvudet, inte svarar på behandling med syrahämmande medicin. Vi har även noterat att de har en sänkt livskvalitet.

De olika delarbetena i avhandlingen har sammantaget visat att inflammation i bakre delen av struphuvudet till följd av reflux, är mindre vanligt än man trott. Patienterna har kvarstående besvär flera år efter det att de blivit insatta på syrahämmande behandling. Detta kan bero på att även om syrahämmande läkemedel sänker surhetsgraden och volymen av reflux, så är antalet refluxer och deras varaktighet, fortfarande lika stort. Dessutom kan det bero på att andra ämnen i magsaften som galla och andra frätande ämnen, fortfarande kan orsaka skada i struphuvudet.

Det finns likheter mellan patienter med inflammation i struphuvudet och patienter med funktionella tarmsjukdomar. Majoriteten av dessa är patienter är kvinnor, med långvariga och varierande besvär och därav följande sänkta livskvalitet. Ytterligare en förklaring till att så få av patienterna i avhandlingen hade reflux trots typiska tecken förenat med symptom på inflammation i struphuvudet skulle kunna vara att de, liksom patienter med funktionell tarmsjukdom, har visceral hypersensitivitet, i svalget. Detta fynd stärks av det faktum att vi funnit antikroppar mot GnRH hos våra patienter, vilket även är vanligt hos patienter med funktionella tarmsjukdomar.

I arbetet med avhandlingen har vi även upptäckt att både patienter med inflammation i bakre delen av struphuvudet och patienter med diabetes, som har av besvär från magen och matstrupen, har förändrade nivåer av hormonet motilin i blodet. Vi har hos patienterna med diabetes också kunnat visa att matstrupens rörlighet är kopplad till nivåerna av motilin i blodet. Detta kan vara ett sätt för kroppen att kompensera den dåliga rörligheten i matstrupen som återfinns hos många av dessa patienter. Hos patienter med inflammation i struphuvudet tror vi att de förändrade motilinnivåerna kan vara en orsak till dålig rörlighet av matstrupen och därmed resultera i reflux.

En del av arbetena i avhandlingen har berört hälsorelaterad livskvalitet. Det finns ett litet antal tidigare studier gjorda när det gäller livskvalitet hos patienter som har inflammation i bakre delen av struphuvudet. Samtliga har visat på sänkt livskvalitet jämfört med friska individer, samt med patienter som besväras av halsbränna. Våra resultat visar att kvinnor, mer än män, påverkas av inflammation i bakre delen av struphuvudet, och att de har påtagligt sänkt livskvalitet jämfört med friska individer, men att deras livskvalitet förbättras efterhand. Det finns forskning som visar att kvinnor, i större utsträckning än män, har en tendens att verbalisera sina kroppsliga besvär, vilket skulle kunna förklara våra resultat. Det faktum att deras livskvalitet förbättras över tid kan bero på att de fått behandling, fått en diagnos samt fått information om sin sjukdom.

När det gäller funktionella tarmsjukdomar finns det medicinsk behandling som kan förbättra patienternas livskvalitet. Då vår forskning visat att patienter med inflammation i struphuvudet har stora likheter med patienter som har funktionella tarmsjukdomar, borde man överväga att prova en del av den behandling som patienter med funktionell tarmsjukdom får, på patienter med symptom på inflammation i struphuvudet. Detta i ett försök att förbättra dessa patienters symptom och livskvalitet.

I korthet har denna avhandling visat att inflammation i struphuvudet kan antas inte endast bero på reflux utan att andra orsaker bör övervägas samt att ny behandling kan/borde övervägas för att förbättra symptom och livskvalitet hos dessa patienter.

Ytterligare forskning kring inflammation i struphuvudet borde inriktas på i vilken utsträckning dålig rörlighet i matstrupen föreligger hos dessa patienter och på vilka ämnen i refluxatet som har negativ inverkan. Vidare bör forskningen inriktas på hur GnRH och antikroppar mot GnRH påverkar visceral hypersensitivitet, rörlighet i matstrupen, samt matstrupens nedre ringmuskel.

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