From the DEPARTMENT OF MEDICAL EPIDEMIOLOGY
AND BIOSTATISTICS
Karolinska Institutet, Stockholm, Sweden

BARRETT'S OESOPHAGUS AND METAPLASIA AT THE OESOPHAGOGASTRIC JUNCTION

AN EPIDEMIOLOGICAL APPROACH

Johan Johansson

Karolinska Institutet

Stockholm 2006

Published and printed by Karolinska University Press
Box 200, SE-171 77 Stockholm, Sweden

ISBN 91-7140-987-4

1 ABSTRACT

The aims of this thesis were to estimate the prevalence of Barrett's oesophagus (BO) and related histological aberrations, to investigate agreement between endoscopy and histology regarding the diagnosis of BO and to investigate risk factors for BO paying particular attention to epidemiological aspects of the study design. We also investigated pancreatic acinar metaplasia (PAM) with special emphasis in its relation to gastro-oesophageal reflux and BO.

Seven hundred and sixty-nine patients endoscoped for the first time at endoscopy units exclusively serving defined catchment areas in southeast Sweden were examined and clinical data recorded. Exposure data were also collected from 160 population controls. In a subsample of 26 patients 24 hour oesophageal pH monitoring were performed.

Overall intestinal (IM) prevalence in the distal oesophagus and/or gastric cardia was 14%. BO was noted in 4%, with a predominance of women (69%). Both prevalence of IM overall and cardia type mucosa in the gastric cardia were significantly associated with increasing age.

Overall concordance between endoscopy and histology regarding columnar mucosa above the oesophagogastric junction was 74% (95% confidence interval [CI] 71-77%) and the agreement beyond chance was fair (Kappa=0.38, 95% CI 0.32-0.45). Our data were consistent with a lower threshold for macroscopic detection of columnar epithelium above the oesophagogastric junction, when risk factors for BO were present.

Reflux symptoms and smoking indicated 10.7- and 3.3-fold risks, respectively, for BO (95% CI 3.5-33.4 and 1.1-9.9, respectively) in the comparison with population controls. Body mass was unrelated to risk. In the cross-sectional analysis among endoscopy room patients, reflux symptoms were associated with an odds ratio (OR) of 2.0 (95% CI 0.8-5.0). This association was, however, modified by the subjunctional presence of *Helicobacter pylori*; although the infection was not in itself importantly connected with risk, a combination of reflux symptoms and *H. pylori* was linked to an almost five-fold risk (95% CI 1.4-16.5), as compared with the absence of both factors.

PAM was found above the oesophagogastric junction (OGJ) in 9% and below the OGJ in 13% (2% had PAM both above and below the OGJ). PAM below and PAM exclusively above the OGJ were both borderline associated with age, with a 2% increase in prevalence per year. PAM exclusively above the OGJ was significantly associated with female gender (OR 2.8, 95% CI 1.3-6.2) and subjunctional presence of *H. pylori* (OR 2.3, 95% CI 1.1-4.9). Among patients with BO 38% had PAM above the OGJ. Mean values for percentage time with oesophageal pH<4.0 indicated pathological gastro-oesophageal reflux among patients who had PAM without and with accompanying BO.

In conclusion, while BO is not very common among Swedish gastroscopy patients, IM and PAM are found in every 7th and 5th patient, respectively. Age-dependent increments in prevalence suggest that not only BO and IM, but also cardia type mucosa, are acquired and/or progressive lesions. The agreement between macroscopic and microscopic assessments of BO is no more than fair, and partly dependent on the presence of patient characteristics suggestive of pathology in the region. Reflux is the dominating risk factor for BO, and proximal gastric colonization of *H. pylori* seems to amplify this risk. PAM might be an age-dependent lesion, associated with *H. pylori,* female gender and gastro-oesophageal reflux if located above the OGJ.

Keywords: Barrett's oesophagus, body mass index, cardia, diagnosis, gastric mucosa, gastroscopy, gastro-oesophageal reflux, *Helicobacter pylori*, histology, metaplasia, oesophagogastric junction, pancreatic acinar metaplasia, risk factors

ISBN 91-7140-987-4

2 CONTENTS

3 LIST OF PAPERS

I. Johan Johansson, Hans-Olof Håkansson, Lennart Mellblom, Antti Kempas, Karl-Erik Johansson, Fredrik Granath, Olof Nyrén.
Prevalence of precancerous and other metaplasia in the distal oesophagus and gastro-oesophageal junction.
Scand J Gastroenterol 2005;40:893-902.

II. Johan Johansson, Hans-Olof Håkansson, Lennart Mellblom, Antti Kempas, Fredrik Granath, Karl-Erik Johansson, Olof Nyrén.
Diagnosing Barrett's oesophagus: factors related to agreement between endoscopy and histology.
Submitted

III. Johan Johansson, Hans-Olof Håkansson, Lennart Mellblom, Antti Kempas, Karl-Erik Johansson, Fredrik Granath, Olof Nyrén.
Risk factors for Barrett's oesophagus: a population-based approach.
Scand J Gastroenterol In press 2006.

IV. Johan Johansson, Hans-Olof Håkansson, Lennart Mellblom, Antti Kempas, Gerhard Kjellén, Lars Brudin, Fredrik Granath, Karl-Erik Johansson, Olof Nyrén.
Pancreatic Acinar Metaplasia in the Distal Oesophagus and the Gastric Cardia prevalence, predictors and relation to GORD.
Manuscript

4 ABBREVIATIONS

BMI	Body mass index
BO	Barrett's oesophagus
CI	Confidence interval
GORD	Gastro-oesophageal reflux disease
IM	Intestinal metaplasia
LSBO	Long segment Barrett's oesophagus
MCM	Macroscopic columnar metaplasia above the oesophagogastric junction visible through the endoscope
NSAID	Non-steroidal anti-inflammatory drug
OGJ	Oesophagogastric junction
OR	Odds ratio
PAM	Pancreatic acinar metaplasia
SSBO	Short segment Barrett's oesophagus
USSBO	Ultrashort segment Barrett's oesophagus

5 INTRODUCTION

There are mainly two driving forces for this thesis work:

1. The essentially unexplained rapidly increasing incidence of oesophageal adenocarcinoma in western societies.
2. The lack of scientifically well-founded management strategies with regard to the clinical management of incidentally found oesophageal columnar metaplasia and Barrett's oesophagus.

Oesophageal adenocarcinoma incidence has increased dramatically in western countries during the last decades (Blot et al. 1991; Powell and McConkey 1992; Hansson et al. 1993; Devesa et al. 1998; Bytzer et al. 1999). In Sweden almost 200 new cases are now diagnosed each year according to the Swedish Cancer Registry (Figure 1), which corresponds to an annual incidence rate of more than 4 per 100 000. In the 1960s the annual incidence rate was less than 1 per 100 000 in Sweden (Hansson et al. 1993). Moreover, the diagnosis is in most cases made in late stage disease when prognosis is poor (Sundelof et al. 2002; Enzinger and Mayer 2003; Shaheen 2005). The strongest known risk factor for oesophageal adenocarcinoma is Barrett's oesophagus, characterized by endoscopically visible columnar mucosa in the distal oesophagus, with histologically confirmed intestinal metaplasia, which also presumably constitutes a necessary step in the causal pathway from normal oesophageal squamous epithelium to adenocarcinoma (Flejou 2005; Lagergren 2005).

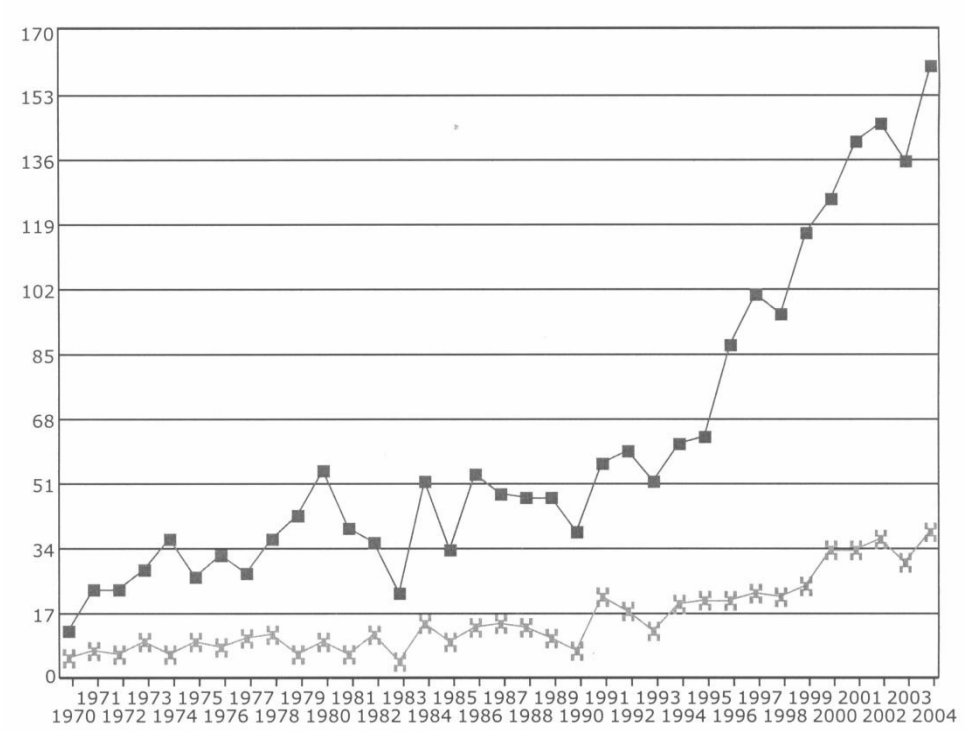

Figure 1. Number of new oesophageal adenocarcinoma cases in Sweden per year. Filled squares denotes men and crosses denotes women (From: The National Board of Health and Welfare, Socialstyrelsen, Statistikdatabaser – Cancerstatistik. Available at: http://www.socialstyrelsen.se/Statistik/statistikdatabas/ (2006-10-25)).

This thesis does not solve the above mentioned two questions. However, understanding the epidemiology of Barrett's oesophagus might shed light on course of events underlying the rising trend in adenocarcinoma incidence. What is the prevalence of Barrett's oesophagus? Which are the risk factors (and causes) of Barrett's oesophagus? If identification of Barrett's oesophagus is a crucial step in preventing adenocarcinoma related death, or even adenocarcinoma development what characterize the diagnostic procedure? What other metaplastic changes in the oesophagogastric junction area may contribute to the understanding of the development of Barrett's oesophagus and adenocarcinoma in this region? These are the questions focused in this thesis.

6 BACKGROUND

6.1 Short history

The Australian born British surgeon Norman Barrett was not the first one to describe columnar epithelium in the distal oesophagus. However, his paper “Chronic peptic ulcer of the oesophagus and "oesophagitis"” from 1950 has become a classic reference in the history of Barrett’s oesophagus (Barrett 1950). In this paper he erroneously described the columnar lined oesophagus as a proximal part of the stomach pulled-up into the chest by scar tissue or by a congenital short oesophagus. A controversy followed over the next few years, when Allison and Johnstone in 1953 argued that the columnar lined intrathoracic structure was really the oesophagus (Allison and Johnstone 1953). In 1957 Norman Barrett eventually agreed to the term “columnar-lined lower esophagus” and subsequently his name became synonymous with the condition (Barrett 1957).

6.2 Rising trends of oesophageal adenocarcinoma and cardia cancer incidence

The most serious consequence of Barrett’s oesophagus is oesophageal malignancy (Falk 2002). From previously being dominated by squamous cell carcinoma related mainly to alcohol consumption and tobacco use oesophageal cancer has since the mid 1970s been characterized by a dramatic increase in adenocarcinoma incidence (Blot et al. 1991; Powell and McConkey 1992; Hansson et al. 1993; Devesa et al. 1998; Bytzer et al. 1999; Botterweck et al. 2000; Powell et al. 2002; Voutilainen and Juhola 2005). In the United States the incidence has increased approximately 300-500% in the last 40 years (Shaheen 2005) and in the UK the increase has been more than 5% per year in the last three decades reaching an incidence of 12-16 per 100 000 person-years (Jankowski et al. 2002). Also stomach cancer has seen a changing pattern where proximal cancers (i.e. cardia cancers) seems to increase, while consistent evidence support a declining incidence of more distal cancers (Hansson et al. 1991; Powell and McConkey 1992; Devesa et al. 1998; Ekstrom et al. 2000; Crew and Neugut 2004). Moreover, the changing incidence trends seem to be restricted to the westernized countries, albeit with certain regional variations (Botterweck et al. 2000; Corley and Buffler 2001; Jankowski et al. 2002; Crew and Neugut 2004).

6.3 Risk factors for oesophageal adenocarcinoma and cardia cancer

Several risk factors for oesophageal adenocarcinoma has been identified, among which the strongest are Barrett’s oesophagus, gastro-oesophageal reflux and obesity (Chow et al. 1998b; Lagergren et al. 1999a; Lagergren et al. 1999b; Farrow et al. 2000; Lagergren 2005). Oesophageal adenocarcinoma is also associated with male sex and, less strongly, with smoking (Lagergren et al. 2000b; Enzinger and Mayer 2003). Medications that relax the lower oesophageal sphincter seem to increase the risk (Lagergren et al. 2000a), while accumulating evidence suggests a protective effect of NSAIDs (Farrow et al. 1998). There is also emerging evidence of an inverse association with *Helicobacter pylori* (Chow et al. 1998a; Ye et al. 2004; de Martel et al. 2005), but the mechanisms remain conjectural. Low intake of fruit, vegetables, and cereal fibres seem to increase the risk of oesophageal adenocarcinoma (Mayne et al. 2001; Terry et al. 2001a; Terry et al. 2001b). Thus, many factors might be involved in the rising incidence of oesophageal adenocarcinoma, but the sex distribution and the prevalence trends of the major risk factors does not match to the characteristics of the cancer, which makes the underlying causes of the rising incidence still poorly understood (Lagergren 2005; Lagergren 2006).

Also with regard to adenocarcinoma of the gastric cardia gastro-oesophageal reflux and obesity are suggested risk factors, albeit considerably weaker (Chow et al. 1998b; Lagergren

et al. 1999a; Lagergren et al. 1999b) and with regard to reflux not as consistent (Farrow et al. 2000). Further, cardia cancer is also a male predominated condition (Crew and Neugut 2004) and associated with smoking (Gammon et al. 1997; Lagergren et al. 2000b). Apparently oesophageal adenocarcinoma and cardia cancer share some epidemiologic features, but one has to take into consideration that there are evidence of a substantial diagnostic mismatch between these tumors (Lindblad et al. 2006).

6.4 Barrett's oesophagus and oesophageal adenocarcinoma

The relationship between Barrett's oesophagus and oesophageal adenocarcinoma has been recognized in numerous publications. In 1952 Morson and Belcher reported adenocarcinoma in a columnar-lined lower oesophagus describing goblet cells adjacent to the carcinoma (Morson and Belcher 1952). This finding was confirmed by others over the following decades (Adler 1963; Hawe et al. 1973; Naef et al. 1975; Haggitt et al. 1978). The size of the cancer risk in patients with Barrett's oesophagus has been a matter of discussion since the 1980s. Risk estimates has been ranging from 0.2% (Cameron et al. 1985) to 2.9% per year (Reid et al. 1992) and evidence of publication bias has complicated the interpretation of the results (Shaheen et al. 2000). During recent years, however, three large cohort studies have emerged reporting oesophageal cancer in Barrett patients at an incidence of 0.4-0.5% per year (Murray et al. 2003; Solaymani-Dodaran et al. 2004; Sharma et al. 2006b).

This increased risk for oesophageal adenocarcinoma in patients with Barrett's oesophagus has brought along clinical guidelines, however debated (Sharma and Sidorenko 2005), recommending that patients diagnosed with Barrett's oesophagus, and suitable for curative surgical treatment of a potential adenocarcinoma (i.e. oesophagectomy), should undergo regular endoscopic surveillance (Walther and Willen 1996; Boyer and Robaszkiewicz 2000; SSAT-AGA-ASGE 2000; Sampliner 2002; BSG 2005). Some experts also advocate screening among certain high-risk groups (Spechler 2002; Eisen et al. 2004).

6.5 Diagnosis and definition of Barrett's oesophagus

Norman Barrett did not include any length criteria for the diagnosis of a columnar lined lower oesophagus and in the 1960s Hayward conveyed the opinion that even the normal oesophagus could be lined by two cm of columnar epithelium (Hayward 1961). In order to avoid false positive diagnoses Skinner et al introduced a three cm criterion in the 1980s (Skinner et al. 1983) and during the following years the diagnosis of Barrett's oesophagus usually included patients with a columnar lined oesophagus (at endoscopy) of at least 3 cm irrespective of histological type of mucosa; three histological types had previously been described in the columnar lined mucosa: gastric-fundic, junctional and specialized columnar epithelium - the latter also called intestinal metaplasia and containing the characteristic goblet cells. (Paull et al. 1976).

Due to the identification of histologically confirmed intestinal metaplasia as the precursor lesion of oesophageal adenocarcinoma (Spechler and Goyal 1996; Falk 2002) the diagnosis of Barrett's oesophagus according to modern standards requires an oesophageal columnar lining of *any* length, supplemented with histologically confirmed intestinal metaplasia (Falk 2002; Sampliner 2002). The three cm rule is, however, still in practice in that patients with intestinal metaplasia in the distal oesophagus often are classified as long ($\geq$ 3 cm) or short ($<$ 3 cm) segment Barrett's oesophagus (Hirota et al. 1999; Gerson et al. 2002), and the insistence on

identification of intestinal metaplasia to establish a diagnosis of Barrett's oesophagus is still debated (BSG 2005).

As realized by Skinner et al the diagnostic procedure harbour several sources of error (Spechler and Goyal 1996; Falk 2002). There are difficulties in adequately assessing the oesophagogastric junction during the endoscopy (hiatal hernia, grade of air insufflation, peristalsis etc.), as well as difficulties in adequately obtaining representative biopsy specimens. Hence there are considerable risks of both false positive and false negative diagnoses (Spechler and Goyal 1996; Oberg et al. 2001; Jones et al. 2002; Coad and Shepherd 2003). In order to standardize the endoscopic diagnosis of Barrett's oesophagus in a reproducible manner an international working group has proposed the "Prague C and M criteria", which are based on the Circular and Maximal extent of the oesophageal columnar mucosa and shown to perform excellent inter-observer agreement (Sharma et al. 2006a).

6.5.1 Diagnostic accuracy

Since the modern diagnosis of Barrett's oesophagus is dependent on *both* endoscopic and histological findings, there is no easily defined gold standard to rely on, when evaluating the diagnostic accuracy. Previous studies have used different endoscopic and histological criteria, with reported sensitivity and specificity figures ranging between 62-92% and 81-96%, respectively (Winters et al. 1987; Woolf et al. 1989; Eloubeidi and Provenzale 1999; Endlicher et al. 2005b). Positive and negative predictive values range between 34-89% and 92-97%, respectively (Woolf et al. 1989; Eloubeidi and Provenzale 1999; Padda and Ramirez 2001; Endlicher et al. 2005b), and the agreement between endoscopy and histology has been reported to around 80-90% (Woolf et al. 1989; Eloubeidi and Provenzale 1999; Endlicher et al. 2005b).

The association between number of biopsies taken from the columnar segment and the positive predictive value of the endoscopic Barrett diagnosis is well recognized (Coad and Shepherd 2003) and there is evidence that the experience of the endoscopist is of importance (Padda and Ramirez 2001). Also the length of the columnar segment is predictive of the yield of intestinal metaplasia on biopsy (Oberg et al. 2001; Csendes et al. 2003). Apart from the sole length of the columnar segment Wallner et al reported correlations between the Z-line appearance and occurrence of intestinal metaplasia at the squamocolumnar junction (the ZAP-classification)(Wallner et al. 2000). How patient characteristics apart from length of the oesophageal columnar lining (and the appearance of the squamocolumnar junction) affect the agreement between endoscopy and histology, with regard to the diagnosis of Barrett's oesophagus, is not thoroughly studied.

Chromoendoscopy and magnification endoscopy are promising alternatives in diagnosing Barrett's oesophagus, however still not sufficiently validated and standardized for routine use (Connor and Sharma 2003).

6.6 Prevalence of Barrett's oesophagus

Divergent results characterize the epidemiology of Barrett's oesophagus. In a review in 1986 Spechler and Goyal indicated that Barrett's epithelium had been observed in 8 to 20% of patients undergoing endoscopic assessment of oesophagitis (Spechler and Goyal 1986). Two studies in the early 1990s reported both a prevalence rate of histologically verified Barrett's oesophagus (columnar mucosa) of 0.7% in patients undergoing upper gastrointestinal

endoscopy for a variety of upper gastrointestinal symptoms (GOSPE 1991; Cameron and Lomboy 1992).

6.6.1 Long and short segment Barrett's oesophagus

While most previous studies have shown a prevalence of long segment, i.e. ≥3 cm, Barrett's oesophagus (LSBO) of approximately 1% among patients having endoscopy irrespective of clinical indication (Spechler et al. 1994b; Cameron 1997; Hirota et al. 1999), short segment Barrett's oesophagus (SSBO) has been reported in 6 to 12% (Spechler et al. 1994b; Hirota et al. 1999; Wolf et al. 2001). In patients with at least weekly heartburn about 5% has been found to have LSBO (Cameron 2002). In one study, however, prevalence rates as high as 7% were reported for LSBO and 17% for SSBO in asymptomatic individuals (Gerson et al. 2002).

Since most publications about Barrett's oesophagus (BO) emanate from specialized centers that may selectively attract patients with known or suspected BO, concerns have been expressed about possible overestimation of BO prevalence among endoscoped patients.

6.6.2 Population prevalence

In 1990 Cameron et al, out from endoscopy and autopsy data, estimated the population prevalence at 0.376 % (95% CI 0.095-0.967) of histologically verified columnar mucosa of ≥ 3 cm in the distal oesophagus (Cameron et al. 1990). Realizing the 5% prevalence of LSBO in patients with frequent heartburn (Cameron 2002) and considering population-based questionnaire data indicating at least weekly gastro-oesophageal reflux symptoms in 20% of the population (Locke et al. 1997), Cameron in a review in 2002 proposed that the prevalence of Barrett's oesophagus in the general adult population can be estimated at about 1 in 100 persons.

Studies including endoscopies in healthy people are ethically problematic and associated with considerable risk of selection bias. In one study, however, a random sample (n=3000) of the adult population was surveyed using a validated questionnaire with a response rate of 74.2% (Ronkainen et al. 2005). Of the responders 1563 were approached to complete the aim of 1000 individuals in whom upper gastrointestinal endoscopy were performed. The endoscopy sample was significantly older than the original random population sample and the youngest age group among the endoscoped reported gastro-oesophageal reflux symptoms significantly more than the questionnaire responders. The prevalence of Barrett's oesophagus (histologically verified specialized intestinal metaplasia in endoscopically suspected columnar-lined oesophagus) in this study was 1.6% (95% confidence interval 0.8-2.4), LSBO 0.5% and SSBO 1.1%. In individuals with troublesome gastro-oesophageal reflux symptoms over the past three months the BO prevalence was 2.3% and 1.2% in those without such symptoms. An endoscopically visible columnar segment of ≥2 cm was recognized in 1.2%.

6.7 Risk factors for Barrett's oesophagus

6.7.1 Barrett's oesophagus and gastro-oesophageal reflux

The prevailing understanding is that Barrett's oesophagus is the result of severe mucosal injury caused by gastro-oesophageal reflux. The damaged squamous oesophageal mucosa is replaced by columnar epithelium (Falk 2002; Spechler 2002). This was shown in a dog experiment already in 1970 (Bremner et al. 1970), and in humans Barrett's oesophagus is associated with gastro-oesophageal reflux symptoms and markers of gastro-oesophageal

reflux disease (Eisen et al. 1997; Voutilainen et al. 2000; Campos et al. 2001; Conio et al. 2002). However, some studies have failed to show any differences between patients with Barrett's oesophagus and patients with severe oesophagitis without Barrett's oesophagus (Neumann and Cooper 1994; Coenraad et al. 1998), and in patients with unknown Barrett's oesophagus (endoscopically diagnosed with symptomatic adenocarcinoma) far from all have a long previous history of reflux symptoms (Cameron 2002). Moreover, in a large population-based study of Barrett's oesophagus prevalence only 56% reported reflux symptoms (Ronkainen et al. 2005). These findings indicate that other factors than gastro-oesophageal reflux contribute to the development of Barrett's oesophagus. One related factor, however, that seems to be of importance is the contents of the refluxate, and several studies have confirmed an association between bile reflux and Barrett's oesophagus (Oberg et al. 2000; Campos et al. 2001; Banki et al. 2005).

6.7.2 Barrett' oesophagus and Helicobacter pylori

Most previous studies show an inverse association with *Helicobacter pylori* (Vicari et al. 1998; Hirota et al. 1999; Voutilainen et al. 2000; Ackermark et al. 2003), albeit not reaching statistical significance in some. One recent study, however, reported higher *H. pylori* prevalence in Barrett's oesophagus patients than in sex-and age-matched controls (Ferrandez et al. 2006).

6.7.3 Other risk factors

Other factors positively associated with Barrett's oesophagus in the previous literature are age (Lieberman et al. 1997; Cameron 1999; Hirota et al. 1999; Voutilainen et al. 2000), male sex (Lieberman et al. 1997; Vicari et al. 1998; Cameron 1999; Hirota et al. 1999; Voutilainen et al. 2000; Campos et al. 2001; Conio et al. 2002), white ethnicity (Hirota et al. 1999), smoking (Eisen et al. 1997; Hirota et al. 1999; Ronkainen et al. 2005) and, less consistent, alcohol use (Ronkainen et al. 2005) and overweight (Caygill et al. 2002; Stein et al. 2005). One study have also reported an association with presence of duodenal ulcer (Conio et al. 2002).

6.8 Intestinal and other metaplasia at the oesophagogastric junction

6.8.1 Intestinal metaplasia at the oesophagogastric junction

The term "intestinal metaplasia at the gastro-oesophageal junction" or ultrashort Barrett's oesophagus (USSBO) has been used to describe the condition when intestinal metaplasia is found in biopsies from a "normal-looking" oesophagogastric junction (ideally when the squamocolumnar junction coincides precisely with the oesophagogastric junction) (Spechler 2004). Intestinal metaplasia (IM) at the oesophagogastric junction (OGJ) is seen in between 6 and 36% of endoscopy patients (Johnston et al. 1996; Nandurkar et al. 1997; Hirota et al. 1999; Voutilainen et al. 1999b). The malignant potential of IM at the OGJ is not clear (Sharma 2001; Spechler 2004) and its aetiology has been intensively debated. Associations with gastro-oesophaeal reflux have been reported on one hand (Oberg et al. 1997a; Oberg et al. 1999) and associations with *Helicobacer pylori* on the other (Goldblum et al. 1998; Goldblum et al. 2002). One author has proposed that IM at the GOJ, rather than constituting an independent condition, represents *either* short segment Barrett's oesophagus *or* IM of the cardia, the latter a consequence of chronic *Helicobacter pylori* gastritis. Others suggest that it is a matter of intestinal metaplasia subtypes (Voutilainen et al. 1999b).

6.8.2 The mucosa in the gastric cardia

Traditionally the normal gastric cardia has been regarded as a narrow circular band, 1.5-3 cm in width, at the transition between the oesophagus and stomach with an epithelium containing tubular glands with mucous-producing cells (Junqueira et al. 1986). This view has been challenged in recent years when some investigators claim that cardia type mucosa in the gastric cardia is acquired, and related to gastro-oesophageal reflux, while "pure oxyntic" (i.e. gastric-fundic) mucosa is the normal lining (Chandrasoma et al. 2000a; Chandrasoma et al. 2000b).

6.9 Pancreatic acinar metaplasia

Another type of metaplasia, resembling pancreatic acinar tissue, has also been identified at the oesophagogastric junction (Krishnamurthy and Dayal 1995; Wang et al. 1996; Polkowski et al. 2000). This type of mucosa, called Pancreatic acinar metaplasia, has been reported in gastric mucosa, at the oesophagogastric junction and in Barrett's oesophagus (Doglioni et al. 1993; Krishnamurthy and Dayal 1995; Wang et al. 1996; Johansson et al. 2005), but the clinical significance is uncertain.

6.9.1.1 Morphology

Pancreatic acinar metaplasia is characterized by cells with basophilic cytoplasm in the basal part, centrally or basally located nucleus and acidophilic granular cytoplasm in the luminal part (Doglioni et al. 1993). Immunohistochemistry shows consistently reactivity for pancreatic lipase and trypsinogen (Doglioni et al. 1993; Wang et al. 1996; Hakansson et al. 2003), it has been shown that pancreatic secretory proteins are produced in pancreatic acinar metaplasia in the distal oesophagus (Hakansson et al. 2003).

6.9.1.2 Prevalence and correlations

The prevalence of pancreatic acinar metaplasia (PAM) in the oesophagogastric junction area is reportedly 18-32% among upper gastrointestinal endoscopy patients (Wang et al. 1996; el-Zimaity et al. 2000; Johansson et al. 2005). Among patients with Barrett's oesophagus pancreatic acinar metaplasia has been found in 7-18% (Krishnamurthy and Dayal 1995; Wang et al. 1996). In one study PAM at the OGJ was associated with mucosal inflammation (Polkowski et al. 2000), but in another study reported to be with no clear relationship to any clinical or histological finding, raising the possibility of a congenital origin (Wang et al. 1996).

In non-cardia gastric mucosa pancreatic acinar metaplasia has been observed in up to 11% of investigated subjects (Doglioni et al. 1993; Wang et al. 1996; Jhala et al. 2003). Pancreatic acinar metaplasia in gastric mucosa has been associated with intestinal and "pyloric type" metaplasia (Doglioni et al. 1993), and with autoimmune gastritis (Jhala et al. 2003).

7 AIMS OF THE STUDIES

- To estimate the prevalence of Barrett's oesophagus and related histological aberrations in a consecutive series of new endoscopy patients representing a geographically defined source population.

- To evaluate the agreement between the endoscopic and microscopic (i.e. at biopsy) assessment of columnar mucosa (and intestinal metaplasia) above the oesophagogastric junction, and the relation between agreement and certain patient characteristics and endoscopic findings, in a routine clinical setting.

- To increase the validity of risk factor data with regard to Barrett's oesophagus.

- To investigate the occurrence of pancreatic acinar metaplasia at the gastro-oesophageal junction and its relation to other clinical characteristics with special emphasis on gastro-oesophageal reflux and Barrett's oesophagus.

8 SUBJECTS AND METHODS

The endoscopy study performed in two county hospitals in Southeast Sweden, which is the backbone in all four papers of this thesis, was originally designed as a pilot (feasibility) study for a large scale investigation of risk factors for oesophageal columnar metaplasia (including Barrett's oesophagus) and determinants for its natural history. In the study we took advantage of some special features characterizing health care in Sweden, and the endoscopy services in the selected study areas in particular. Since practically all hospital-linked health care is delivered at tax-financed public institutions run by the county councils, and since patients have been traditionally obliged to use the hospitals in their county of residence when in need of such medical services, hospital-linked care has, in practice, been population-based and referable to the county where the patient lives.

In the pilot study (from March to June 1997, and from April 1998 to March 1999 – new extended patient recruitment) five endoscopy centres (three county hospitals and two university hospitals) were initially included in the prospective recruitment of consecutive gastroscopy patients. Inclusion criteria were "first-time" upper endoscopy (i.e. no upper endoscopy in the past three years, and a new clinical indication, according to the patient's case record) and age 18-79 years. The recruitment process with regard to appropriate selection of consecutive patients was deemed acceptable only in two centres (Kalmar and Växjö, both primary referral centres), which were selected for the analyses of prevalence, risk factors and diagnostic accuracy.

8.1 Endoscopy (I-IV)

The oesophagus, oesophagogastric junction (OGJ) and upper stomach of all participants were examined according to a standardized protocol. The endoscopies were performed or supervised by experienced endoscopists. Recorded were: indication for the endoscopy, distance between the incisors and the OGJ (defined as the point where the tubular oesophagus meets the gastric folds), occurrence and length of hiatal hernia, severity of oesophagitis (according to Savary-Miller grade I-IV(Miller 1992)), occurrence and anatomic distribution of columnar epithelium proximal to the OGJ, and other abnormal findings in the oesophagus, stomach and duodenum. Biopsies were taken immediately below and above the OGJ, two at each site. If macroscopic columnar metaplasia was observed in the distal oesophagus, two biopsies were obtained every second cm upwards until normal squamous epithelium was reached (Figure 1).

8.2 Histological evaluation (I-IV)

Biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin, sectioned at 4-5 levels and stained with van Gieson. Furthermore, all slides were stained with alcian blue – PAS, but only examined in equivocal cases. All biopsies were examined by the same pathologist (L.M.), who was blinded to the endoscopic findings. A standardized protocol was used for registration of number and quality of the biopsies, type of mucosa (squamous, gastric-fundic, cardiac or intestinal), occurrence of inflammation and atypia/dysplasia/cancer. Intestinal metaplasia (IM) was defined by the presence of at least one unequivocal goblet cell on routine sections. Cardiac mucosa was defined as at least one lobular gland composed only of mucous producing cells of cardiac type. Gastric-fundic type mucosa was defined as glands containing parietal and chief cells. Biopsies with more than one type of glands were classified according to the most pathologic type of gland in the following order: IM, cardiac and gastric-fundic or squamous depending on biopsy site. Whenever IM was found, cardiac mucosa was also present. A reassessment was done (by the same pathologist) of all preparations with IM or cardiac mucosa for registration of pancreatic acinar metaplasia (PAM), and of all

preparations with active inflammation (≥3 intraepithelial granulocytes in at least one high power field; 0.148 mm²) in the cardia for registration of *Helicobacter pylori* in biopsies below the GOJ using a 2% Giemsa stain. PAM was defined as glandular cells with basophilic cytoplasm in the basal part, centrally placed nucleus and pikrinophilic granular cytoplasm in the luminal part. Since PAM was not observed in specimens with only gastric-fundic type or only squamous epithelium, these biopsies were not reassessed.

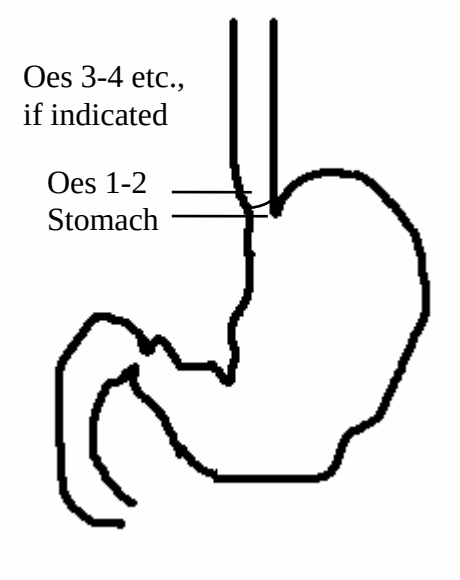

Figure 1. Figure included in the standardized endoscopy protocol. Two biopsies were taken below the oesophagogastric junction (within 2 cm, Stomach) and two biopsies immediately above (within 2 cm, Oes1-2) in each patient. If macroscopic columnar metaplasia was observed, two biopsies were obtained every second cm upwards until normal squamous epithelium was reached (Oes 3-4 etc.).

8.3 Diagnostic criteria for Barrett's oesophagus

Barrett's oesophagus (BO) was defined as concomitant presence of endoscopically visible columnar mucosa of any length and histologically confirmed intestinal metaplasia above the oesophagogastric junction. The combination of short-segment (<3 cm) columnar mucosa and intestinal metaplasia was referred to as short-segment Barrett's oesophagus (SSBO), whereas the combination of long-segment (≥3 cm) columnar mucosa and intestinal metaplasia was referred to as long-segment Barrett's oesophagus (LSBO).

8.4 Questionnaire (I-IV)

The patients filled in a questionnaire regarding body height, weight, reflux symptoms, tobacco use, snuff dipping, alcohol use, heredity and medication. Heartburn and/or acid regurgitation > 50 times/year was regarded as positive for reflux symptoms.

8.5 Population controls (III-IV)

We supplemented our endoscopy room-based cross-sectional study subjects with a second control group recruited through a stratified random sample (not endoscoped) from the source populations, frequency matched according to age and sex with the cases found to have intestinal metaplasia in the distal oesophagus.

8.6 24 hour oesophageal pH monitoring

The method of 24 hour pH monitoring has previously been described (Kjellen et al. 1991). In summary, a portable pH recording equipment from Synectics© was used comprising an antimony pH electrode placed 5 cm above the cardia and, as reference, a silver-silver chloride cutaneous electrode on the chest after having been calibrated with buffer solutions (pH 7.0 and 1.0). The electrodes were connected to a solid state memory unit. The patients were told to avoid acid liquids and long-acting anti-reflux medication 48 h before the investigation and to record erect and supine periods. The data were analysed with a standard computer program (Esophagogram, Synectics; IBM PC). The reference value of pH below 4 during the 24 h registration is 3.4% for total time (Johnsson et al. 1987). Limits for supine time and upright time are 1.2% and 6.3%, respectively (Kjellen et al. 1991). The Symptom Index (SI) was calculated by the following formula: (number of symptoms correlated to a pH value <4 / total number of symptoms) x 100 (Wiener et al. 1988).

8.7 Statistical analysis

8.7.1 Prevalence rates, proportions and kappa statistics (I-II,IV)

In **study I** crude prevalence rates were calculated by dividing the number of subjects with positive findings by the number investigated for this finding (i.e. all patients with number of biopsies strictly according to protocol [at least two biopsies on each level] plus all patients with a positive finding [of the mucosa of investigation] in any biopsy). In **study IV** we restricted the prevalence calculations exclusively to patients with number of biopsies strictly according to protocol (at least two biopsies on each level). Ninety five percent exact binomial confidence intervals (CI) were computed by means of JavaStat -- Binomial and Poisson Confidence Intervals (http://members.aol.com/johnp71/confint.html). In **study II** proportions (including concordance rates) with 95% exact binomial confidence intervals (CI) were computed by means of JavaStat -- Binomial and Poisson Confidence Intervals (http://members.aol.com/johnp71/confint.html). The kappa statistics with 95% confidence intervals (CI) were computed by means of the FREQ procedure in SAS 8.02 (SAS Institute Inc., Cary, NC, USA). Agreement according to kappa was classified as follows: <20 poor, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.80 good and 0.81-1.00 very good.

Medians and means, with 95% CIs, were calculated according to standard methods (Bland 2000).

8.7.2 Univariable comparisons

Age differences were tested with two-sided Two-Sample t-Test for Means and two-sided Wilcoxon ranksum test where appropriate. Differences in population proportions (including concordance rates) were tested with two-sided Two-Sample Test for Proportions (z test) or two-sided Fisher's Exact Test where appropriate. SAS. Differences between different kappas (stratum-specific) were tested by means of the likelihood score method (Nam 2006). A p-value <0.05 was regarded as significant.

8.7.3 Multivariable comparisons

In **study I** associations, expressed as odds ratios (OR) with 95% confidence intervals (CI), of age, gender, reflux symptoms, BMI, smoking and *Helicobacter pylori* with PAM (or cardiac type mucosa) were modeled with multivariable logistic regression. The other odds ratios were adjusted for age and sex unless otherwise stated in the text.

In **study III** and **IV** associations, expressed as odds ratios (OR) with 95% confidence intervals (CI) with age (continuous), gender, reflux symptoms (yes/no), body mass index (BMI, tertiles), *H. pylori* (present/absent), smoking (ever/never), and alcohol consumption (user/abstainer) were modelled with multivariable logistic regression. Interaction (**study III)** was evaluated by including product terms in the models, and the statistical significance on the 5% level was tested by likelihood ratio tests (Hosmer and Lemeshow 1989).

In **study III** we performed separate analyses for 3 partly overlapping outcome categories: (i) Macroscopic columnar metaplasia (MCM) above the GOJ, visible through the endoscope; (ii) histologically confirmed IM above the GOJ; and (iii) Barrett's oesophagus, defined as concomitant presence of MCM of any length and IM above the GOJ.

All multivariable statistical analyses were performed using SAS; PROC LOGISTIC (**study I**) or PROC GENMOD (**study III-IV**) was used for the logistic regression modelling (SAS Institute Inc., Cary, NC, USA).

9 RESULTS

9.1 Subject participation

9.1.1 Endoscopy

During the study periods, 1191 eligible patients underwent endoscopies at the two endoscopy services. For another 364 endoscopies, we were unable to establish eligibility since the staff failed to complete the study protocol. Since, overall, slightly less than half of the patients at the units under study were eligible, we estimate that some 170 out of the 364 missed patients should have been included. A further 403 eligible patients evaded inclusion for various reasons (Figure 2). Thus, the participation rate was approximately 788/1361 (58%). Among the 788 participants 19 patients had not evaluable biopsies.

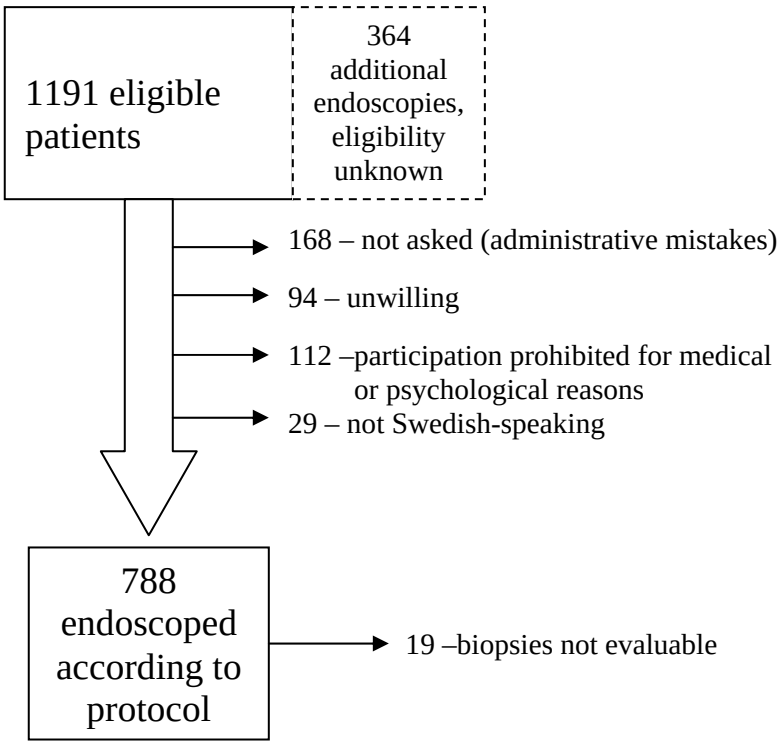

Figure 2. Flow chart for eligible patients and reasons for non-participation.

9.1.1.1 Comparison between participants and non-participants

To evaluate the possible impact of selection factors introduced through non-participation, we compared participants with eligible patients who did not participate, and with patients with unknown eligibility, with regard to background factors, including indications for endoscopy and main findings (Table 1). We scrutinized the endoscopy registry at one of the two Hospitals (Kalmar), and information was retrieved manually from case record forms. We found only small differences in age and gender distribution, with the study population being slightly younger and the eligible non-participants slightly more female predominated than the

rest of the patients. An ulcer indication as well as ulcer diagnosis was less common among participants than non-participants. However, the potentially most important difference was a considerably higher proportion with suspicion of reflux/oesophagitis as the main indication among participating patients, compared to non-participants (40% among participants versus

Table 1. Demographics and clinical data of participants and non-participants

Feature		Study population (n=769)	Eligible non-participants (n=422)	Non-participants with unknown eligibility (n=364)
Age(yrs)	mean	53	55	56
	median	54	56	58
Gender	males	43%	39%	43%
	females	57%	61%	57%
Main indication for the endoscopies				
Suspicion of GORD[1]/ oesophagitis		40% (304/769)	18% (56/305)	11% (25/235)
Suspicion of peptic ulcer		37% (282/769)	40% (121/305)	47% (110/235)
Suspicion of malignancy		5% (41/769)	10% (32/305)	12% (28/235)
Diagnosis at endoscopy				
Hiatal hernia[2]		24% (184/765)	12% (29/244)	4% (11/274)
Oesophagitis[3]		18% (141/767)	12% (28/242)	10% (28/274)
Gastric ulcer		4% (27/652)	7% (16/225)	8% (22/274)
Duodenal ulcer		4% (28/654)	7% (16/225)	5% (14/274)
Malignant tumor				
	Oesophagus	0% (0/700)	0% (0/225)	0.4% (1/274)
	Stomach	0.2% (1/652)	0.4% (1/225)	0.4% (1/274)
Symptoms of GORD (heartburn and/or acid regurgitation)				
> 50 times/year		33% (224/682)	no information	no information
< 3 times/year		17% (119/682)	no information	no information

[1]GORD, gastro-oesophageal reflux disease
[2]Hiatal hernia, distance >2 cm between diaphragmatic hiatus and GOJ
[3]Oesophagitis, Savary-Miller grade I-IV
Within parenthesis numbers/total numbers providing retrievable information

18% among established eligible non-participants, and 15% among all non-participating patients). Further, oesophagitis was more common as the main finding among participants than among non-participants (18% versus 12% and 10%).

9.1.2 Histology

9.1.2.1 Study I

Prevalence calculations were made in 690, 747 and 753 for intestinal metaplasia, gastric-fundic versus cardiac mucosa in the cardia and pancreatic acinar metaplasia, respectively.

9.1.2.2 Study II

Agreement regarding columnar mucosa was calculated in 705 patients with at least two biopsies above the OGJ and/or any biopsy positive for columnar mucosa above the OGJ. Agreement with regard to endoscopic finding versus histologically confirmed intestinal metaplasia was calculated in 697 patients with at least two biopsies above the OGJ and/or any biopsy positive for intestinal metaplasia above the OGJ.

9.1.2.3 Study IV

Six hundred and forty-four patients were included in the prevalence calculations

9.1.3 Questionnaire

9.1.3.1 Study I

In the multivariable analysis with regard to cardiac mucosa in the gastric cardia and with regard to PAM below and/or above the OGJ 621 and 633 patients with complete questionnaire data were included, respectively.

9.1.3.2 Study II

In the analyses regarding columnar mucosa, and regarding intestinal metaplasia, stratified by reflux symptoms 614 and 609 patients with complete questionnaire data were included, respectively. In similar analyses stratified by body mass index 615 and 610 patients were included, respectively.

9.1.3.3 Study III-IV

In the multivariable analyses with regard to MCM, IM above the OGJ, Barrett's oesophagus and PAM 604 patients and 160 population controls (53 % of selected, 160/300) were included.

9.1.4 Population controls

160 population controls (53% of the 300 randomly selected, participation among men was 50% and among women 55%). We compared quick responders (n=112) with reluctant ones (n=44), assuming that the latter were more similar to the non-participants. The prevalence of reflux symptoms was 8% and 16% among quick and reluctant responders, respectively. Hence, it appears that the non-participation might have led to some underestimation of the prevalence of reflux symptoms in the population controls.

9.1.5 24 hour pH monitoring

Forty nine of the study patients (with questionnaire information on reflux symptoms and still alive) from one centre (Kalmar) were invited to a supplementary 24 hour oesophageal pH

monitoring: 30 randomly selected patients (out of 42 eligible) with PAM above the OGJ and no Barrett's oesophagus, all 7 patients with Barrett's oesophagus *and* PAM above the OGJ and all 12 patients with Barrett's oesophagus *without* PAM above the OGJ. Twenty-six patients (53%) were included.

9.2 Prevalence data (Paper I,IV)

9.2.1 Endoscopic findings

The main diagnoses made upon endoscopy are listed in Table 1. Oesophagitis grade III-IV was found in 3% (95% CI 2-5%). Endoscopic columnar epithelium in the oesophagus was found in 17% (95% CI 15-20%). Mean and median lengths of the columnar epithelium were 1.8 cm and 1.0 cm, respectively, and reported as circumferential in 58%. Columnar epithelium with a length equal to or exceeding 3 cm was noted in 24 patients (3%, 95% CI 2-5%). One case of cardia cancer was found, but no oesophageal cancer.

9.2.2 Histological findings

The mucosa types observed above and immediately below the OGJ in patients without and with endoscopically visible columnar epithelium in the oesophagus are shown in Figure 3. The numbers are based on 741 patients with biopsies that could be adequately evaluated, but owing to missing information on histology from either of the anatomic levels in a few patients, the totals in the distributions by histology are slightly lower than the totals for the endoscopic assessments. The findings below the OGJ (i.e. in the cardia) were similar for patients with and without visible oesophageal columnar epithelium. Immediately above the OGJ among the patients without visible columnar epithelium, 73% had regular squamous epithelium, 21% had cardiac mucosa without intestinal metaplasia (IM), 4% had gastric-fundic mucosa, and 2% had IM. As expected, these figures were different among the patients with endoscopically visible columnar epithelium: 50% had cardiac mucosa without IM, 11% had gastric-fundic mucosa, 14% had squamous epithelium and 25% had IM. The prevalence of the different types of mucosa by level above the OGJ among patients with endoscopically visible columnar epithelium is shown in Table 2.

Histologic evidence of *H. pylori* colonization in the proximal stomach was found in 19% (140) of the patients. Apart from the cardia cancer mentioned above, three patients had low-grade dysplasia in the cardia and one patient had low-grade dysplasia in the oesophagus. None of the patients had high-grade dysplasia.

9.2.3 Intestinal metaplasia and Barrett's oesophagus

Overall, 100 patients (14%, 95% CI 12-17%) had IM in the distal oesophagus and/or in the cardia. The gender distribution among these patients was identical to that among all participating patients (43% males), indicating that among patients coming for first-time endoscopy the prevalence is similar for both sexes. The mean age among patients with IM was 64 years. The prevalence increased with age (Figure 4) from 2% among the youngest to 33% among the oldest (OR 1.08 per year, 95% CI 1.06-1.10, similarly for men and women). Of the patients without endoscopically visible columnar metaplasia in the oesophagus, 64 (11%, 95% CI 9-14%) had IM in biopsies from the cardia and/or distal oesophagus (IM-OGJ), with a slightly higher male/female ratio (47/53) but similar relation to age (mean age 65 years, OR 1.07, 95% CI 1.04-1.11 and OR 1.10, 95% CI 1.06-1.14 per year for men and women, respectively) as in the total material.

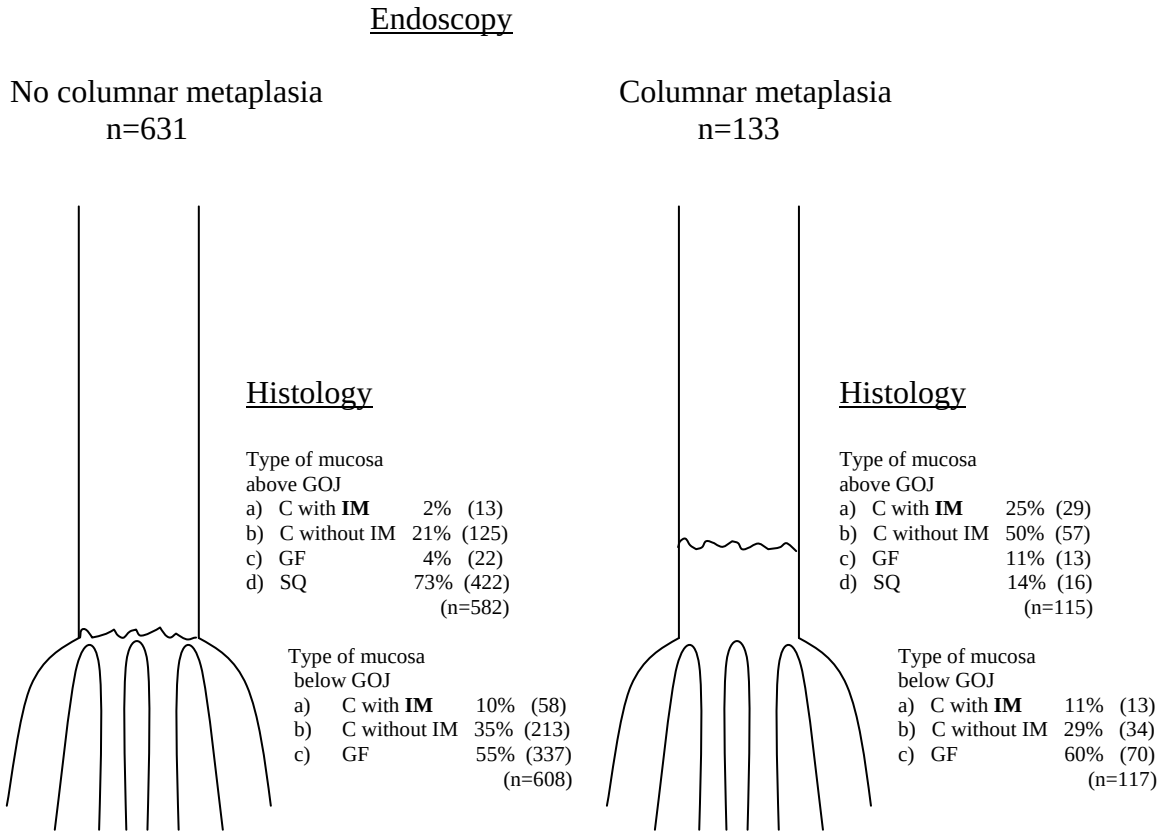

Figure 3. Mucosa types above and immediately below the gastro-oesophageal junction (GOJ) in patients without and with macroscopically visible columnar epithelium in the oesophagus. Due to missing information on histology from either of the anatomic levels in a few patients, the totals in the distributions by histology are slightly lower than the totals for the endoscopic assessments. IM=intestinal metaplasia (always located in cardia type mucosa), C=cardiac type mucosa/metaplasia, GF=gastric-fundic type mucosa/metaplasia and SQ=squamous epithelium.

Intestinal metaplasia was observed in 23 (23%, 95% CI 15-32%) out of 101 patients with short segment (<3 cm) and 6 (43%, 95% CI 18-71%) out of 14 with long-segment (≥3 cm) columnar epithelium (p=0.11). Hence, the prevalence of SSBO and LSBO were 3% (23/747, 95% CI 2-5%) and 0.8% (6/747, 95% CI 0.3-1.7%), respectively. There was a female predominance (65%) among SSBO patients exceeding that in the entire material, indicating a higher probability of SSBO among women coming for first-time endoscopy, compared to men. The mean age among SSBO patients was 59 years. No fewer than 5 out of 6 LSBO patients were women, and the mean age was 67 years. Both SSBO and LSBO patients were significantly older than those without these lesions (p=0.03 and p=0.02, respectively). The mean age among all 29 patients with Barrett's oesophagus was 61 years (median 64).

Table 2. Prevalence of intestinal metaplasia, cardiac mucosa, gastric-fundic mucosa and squamous epithelium by level above the oesophagogastric junction (OGJ) among patients with endoscopically visible columnar epithelium in the oesophagus.

Type of mucosa*	Level above GOJ					
	0-2 cm		>2 and ≤4 cm		>4 cm	
	%	(n:o)	%	(n:o)	%	(n:o)
intestinal metaplasia	18	(21/116)	12	(14/116)	0.9	(1/115)
cardiac	51	(59/116)	16	(18/116)	2	(2/115)
gastric-fundic	16	(18/116)	3	(4/116)	0	(0/115)
squamous	16	(18/116)	69	(80/116)	97	(112/115)

*In some patients the specified type of mucosa was found at more than one level. The prevalence of intestinal metaplasia at any level above the GOJ was 25% (29/115), and corresponding figures for cardiac, gastric-fundic and squamous epithelium were 50% (57/115), 11% (13/115) and 14% (16/115), respectively.

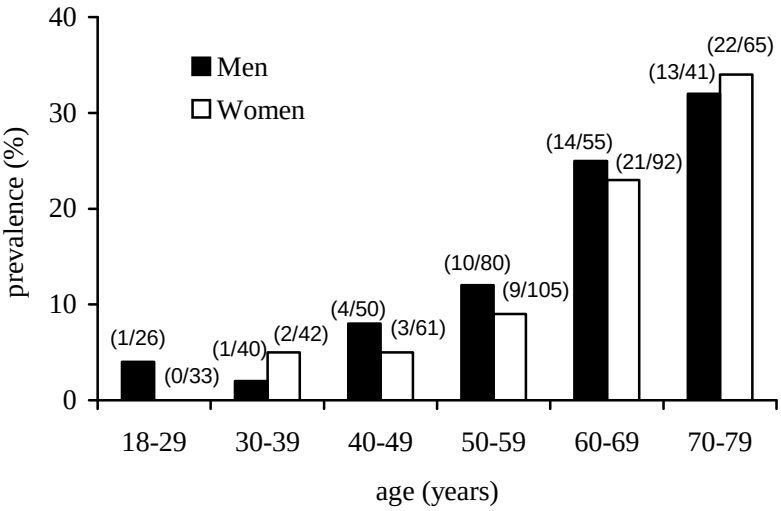

Figure 4. Prevalence of intestinal metaplasia (IM) overall in the cardia and distal oesophagus by age and sex.

To investigate the impact of the selection bias introduced through non-participation, we re-calculated our prevalence figures stratified for indication (304 patients with and 465 without suspicion of reflux/oesophagitis as the main indication). In these stratified analyses, the overall prevalence of IM was 15% and 14% respectively, IM-GOJ 12% and 11% and SSBO 3% in both strata of patients with and without suspicion of reflux/oesophagitis as the main indication. The prevalence of LSBO (3/291 and 3/456) was too low to allow meaningful stratification.

9.2.4 Gastric-fundic versus cardiac mucosa in the cardia

In the cardia (immediately below the OGJ) we found cardiac mucosa, with or without IM, in 44% (327 patients, 95% CI 40-47%) of the patients, and in the remaining 56% (95% CI 53-60%) we found only gastric-fundic mucosa. The prevalence of cardiac mucosa increased with age (OR 1.03 per year adjusted for gender, 95% CI 1.02-1.04, similarly for men and women) (Figure 5) and the mean age (57 years) among these patients was significantly higher ($p<0.0001$) than among those with only gastric-fundic mucosa (51 years). The proportion of women (51%) among patients with cardiac mucosa was slightly lower than that among all patients, reflecting a somewhat higher prevalence among participating men compared to women (50% versus 39%, $p=0.004$).

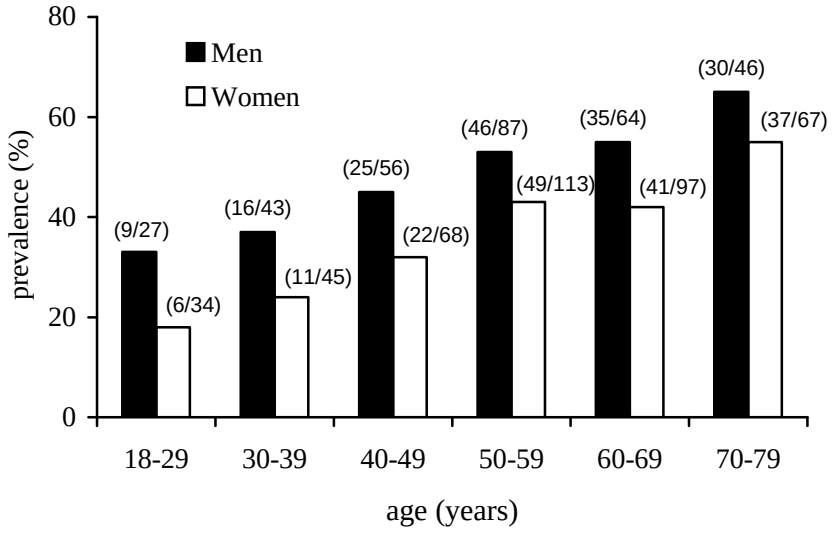

Figure 5. Prevalence of cardiac type mucosa in the cardia by age and sex.

9.2.5 Pancreatic acinar metaplasia

PAM was seen exclusively in patients with cardiac mucosa or intestinal metaplasia, and we found no single case with this metaplasia in specimens containing only gastric-fundic or squamous epithelium. Six hundred and forty four patients investigated strictly according to protocol (at least two biopsies on each level) were included in the prevalence calculations (**study IV**). Mean age was 53 years and 43% were men. PAM was found in any biopsy above and/or below the OGJ in 121 patients with a mean age of 55 years and a proportion of women (55%) comparable to that among all study participants. In most cases PAM was located either

above *or* below the OGJ; hence, 8% (95% CI 6-11%) had PAM above the OGJ and 13% (95% CI 10-15%) of the patients had PAM below the OGJ. Only 2% (95% CI 1-4%) had PAM in biopsies *both* above and below the OGJ. Out of 21 patients who fulfilled the diagnostic criteria for Barrett's oesophagus (i.e. endoscopically visible columnar mucosa of any length with histologically confirmed intestinal metaplasia above the OGJ) 8 (38%, 95% CI 18-62%) had PAM above the OGJ. Among 78 patients with endoscopically visible columnar mucosa in the distal oesophagus (any length), regardless of histology, 22 (28%) had PAM above the OGJ and among patients with a length of ≥ 3 cm of such mucosa this proportion was 1/8. In patients with any histological columnar metaplasia (fundic, junctional or intestinal type) in biopsies above the OGJ, regardless of the endoscopic findings, 23% (54 out of 230) also had PAM in these biopsies.

9.3 Diagnostic accuracy (Paper II)

9.3.1 Agreement regarding presence of columnar mucosa

One hundred and seventeen patients (17%, 95% CI 14-20%) had endoscopically visible columnar mucosa above the oesophagogastric junction (mean length 2 cm, median 1 cm, range 1-8 cm), and in 267 patients (38%, 95% CI 34-42%) we found columnar mucosa (gastric-fundic, cardiac, or specialized intestinal) in at least one biopsy above the oesophagogastric junction. The distribution of patients according to results of the endoscopic and histological assessments overall is presented in Table 3. In 74% of the patients (95% CI 71-77%) the two assessments were concordant. The overall agreement beyond chance (kappa [K] statistic)(Altman 1991) was classified as fair (K=0.38, 95% CI 0.32-0.45)(Table 4).

Table 3. Results of endoscopic and histological assessment of columnar mucosa above the oesophagogastric junction overall (n=705). Grey fields signify concordant assessments.

		Microscopic columnar mucosa	
		Yes	No
Endoscopic columnar mucosa	Yes	101 (14%)	16 (2%)
	No	166 (24%)	422 (60%)

9.3.1.1 Impact of patient characteristics

In order to evaluate the impact of patient characteristics on the agreement between the endoscopic and histological assessments of columnar mucosa above the oesophagogastric junction we stratified the patients by gender, age, body mass index, presence of reflux symptoms, hiatal hernia, and oesophagitis. Presence of hiatal hernia significantly (p=0.02)

modified the concordance rate; this rate was 81% (95% CI 74-87%) among patients with hiatal hernia at endoscopy compared to 72% (95% CI 68-76%) for patients without such a hernia (Table 5). The increase was mainly attributable to a lower degree of endoscopic underascertainment of histologically confirmed columnar mucosa; the proportion who were endoscopically positive doubled (13% vs. 27%), while the prevalence of histologically positive cases remained unchanged. Although similar shifts towards higher proportions of positive findings by endoscopy were noted among older compared to younger patients, and among patients with reflux symptoms or oesophagitis compared to those without, the degree of endoscopic underascertainment of histologically confirmed columnar mucosa remained essentially unaltered. Hence, the concordance rate did not differ significantly between these strata. However, high body mass index (≥25), compared with low (<25), was associated with significantly (p=0.03) better concordance (79% [95% CI 74-83%] versus 71% [95% CI 66-76%]).

Table 4. Kappa values for the agreement between the endoscopic and the histological assessment of columnar mucosa above the oesophagogastric junction.

	Values of kappa	95% CI	
Overall	0.38	0.32-0.45	
Age 18-50	0.32	0.21-0.42	p=0.19
Age 51-79	0.42	0.34-0.50	
Men	0.37	0.27-0.48	p=0.95
Women	0.39	0.31-0.47	
Patients without hiatal hernia[1]	0.32	0.25-0.39	p=0.0005
Patients with hiatal hernia[1]	0.58	0.45-0.71	
Patients without oesophagitis[2]	0.36	0.29-0.44	p=0.47
Patients with oesophagitis[2]	0.42	0.28-0.57	
Patients without reflux symptoms[3]	0.33	0.25-0.42	p=0.06
Patients with reflux symptoms[3]	0.49	0.37-0.61	
Body mass index < 25	0.33	0.23-0.42	p=0.02
Body mass index ≥ 25	0.48	0.38-0.58	

[1]Hiatal hernia, distance >2 cm between diaphragmatic hiatus and the oesophagogastric junction
[2]Oesophagitis, Savary-Miller grade I-IV
[3]Reflux symptoms, heartburn and/or acid regurgitation > 50 times/year

Table 5. Results of endoscopic and histological assessment of columnar mucosa above the oesophagogastric junction stratified by age, gender, presence of hiatal hernia, oesophagitis, reflux symptoms and overweight (body mass index ≥ 25). Grey fields signify concordant assessments.

Stratification variable			**Microscopic** columnar mucosa			
Age (yrs)			Yes	No	Yes	No
18-50 (left, n=281) versus 51-79 (right, n=424)	**Endoscopic** columnar mucosa	Yes	29 (10%)	4 (1%)	72 (17%)	12 (3%)
		No	71 (25%)	177 (63%)	95 (22%)	245 (58%)
Gender						
Men (left, n=299) versus women (right, n=406)	**Endoscopic** columnar mucosa	Yes	38 (13%)	8 (3%)	63 (16%)	8 (2%)
		No	66 (22%)	187 (63%)	100 (25%)	235 (58%)
Hiatal hernia[1]						
Absence (left, n=544) versus presence (right, n=161)	**Endoscopic** columnar mucosa	Yes	63 (12%)	10 (2%)	38 (24%)	6 (4%)
		No	142 (26%)	329 (60%)	24 (15%)	93 (58%)
Oesophagitis[2]						
Absence (left, n=579) versus presence (right, n=126)	**Endoscopic** columnar mucosa	Yes	70 (12%)	11 (2%)	31 (25%)	5 (4%)
		No	135 (23%)	363 (63%)	31 (25%)	59 (47%)
Reflux symptoms[3]						
Absence (left, n=409) versus presence (right, n=205)	**Endoscopic** columnar mucosa	Yes	49 (12%)	10 (2%)	39 (19%)	5 (2%)
		No	100 (24%)	250 (61%)	41 (20%)	120 (59%)
Overweight						
Absence (left, n=300) versus presence (right, n=315)	**Endoscopic** columnar mucosa	Yes	39 (13%)	7 (2%)	52 (17%)	9 (3%)
		No	79 (26%)	175 (58%)	57 (18%)	197 (63%)

[1]Hiatal hernia, distance >2 cm between diaphragmatic hiatus and the oesophagogastric junction;
[2]Oesophagitis, Savary-Miller grade I-IV;
[3]Reflux symptoms, heartburn and/or acid regurgitation > 50 times/year
Some of the total percentages in the tables do not equal 100 due to rounding off.
Analyses stratified by reflux symptoms and body mass index were only undertaken in 614 and 615 patients, respectively, for whom adequate questionnaire information was available.

Statistically significant variation in agreement beyond chance (K) was noted between presence and absence of hiatal hernia (p=0.0005, better among patients with hiatal hernia) and presence and absence of overweight (BMI ≥25, p=0.02, better among overweight patients). Borderline significant variation was noted between presence and absence of reflux symptoms (p=0.06, better among those with reflux symptoms) (Table 4).

9.3.2 Endoscopic finding versus histologically confirmed intestinal metaplasia

Six hundred and ninety seven patients could be adequately evaluated regarding intestinal metaplasia in biopsies above the oesophagogastric junction, together with complete endoscopy data. Forty two patients (6%, 95% CI 4-8%) had intestinal metaplasia above the oesophagogastric junction. The concordance between the endoscopic assessment of columnar mucosa and histological assessment of intestinal metaplasia was 86% (95% CI 83-88%) (Table 6). The K value was 0.31 (95% CI 0.21-0.41). Concordance rates calculated on stratified data revealed that the evaluations were less concordant in older patients than in younger (83% [95% CI 79-87%] versus 90% [95% CI 86-93%]), less concordant in patients with hiatal hernia than in those without (78% [95% CI 70-84%] versus 88% [95% CI 85-91%], and less concordant in patients with oesophagitis than in those without (76% [95% CI 67-83%] versus 88% [95% CI 85-91%])(Table 7). All these differences were highly significant (p=0.01, p=0.0007 and p=0.0004, respectively). The greater discordance was attributed mainly to a tendency towards endoscopic overdiagnosis relative to the histological diagnosis of intestinal metaplasia in these strata. Although K values tended to be lower in these strata, none of these differences attained statistical significance (data not shown).

Table 6. Results of endoscopic assessment of columnar mucosa and histological assessment of intestinal metaplasia above the oesophagogastric junction overall (n=697). Grey fields signify concordant assessments.

		Microscopic *intestinal metaplasia*	
		Yes	No
Endoscopic columnar mucosa	Yes	29 (4%)	86 (12%)
	No	13 (2%)	569 (82%)

Table 7. Results of endoscopic assessment of columnar mucosa and histological assessment of intestinal metaplasia above the oesophagogastric junction stratified by age, gender, presence of hiatal hernia, oesophagitis, reflux symptoms and overweight (body mass index ≥ 25). Grey fields signify concordant assessments.

Stratification variable			**Microscopic** *intestinal metaplasia*			
Age (yrs)			Yes	No	Yes	No
18-50 (left, n=276) versus 51-79 (right, n=421)	**Endoscopic** columnar mucosa	Yes	6 (2%)	26 (9%)	23 (5%)	60 (14%)
		No	2 (1%)	242 (88%)	11 (3%)	327 (78%)
Gender						
Men (left, n=295) versus women (right, n=402)	**Endoscopic** columnar mucosa	Yes	9 (3%)	36 (12%)	20 (5%)	50 (12%)
		No	6 (2%)	244 (83%)	7 (2%)	325 (81%)
Hiatal hernia[1]						
Absence (left, n=536) versus presence (right, n=161)	**Endoscopic** columnar mucosa	Yes	19 (4%)	52 (10%)	10 (6%)	34 (21%)
		No	11 (2%)	454 (85%)	2 (1%)	115 (71%)
Oesophagitis[2]						
Absence (left, n=574) versus presence (right, n=123)	**Endoscopic** columnar mucosa	Yes	21 (4%)	59 (10%)	8 (6%)	27 (22%)
		No	10 (2%)	484 (84%)	3 (2%)	85 (69%)
Reflux symptoms[3]						
Absence (left, n=407) versus presence (right, n=202)	**Endoscopic** columnar mucosa	Yes	14 (3%)	45 (11%)	10 (5%)	32 (16%)
		No	10 (2%)	338 (83%)	2 (1%)	158 (78%)
Overweight						
Absence (left, n=298) versus presence (right, n=312)	**Endoscopic** columnar mucosa	Yes	12 (4%)	34 (11%)	14 (4%)	45 (14%)
		No	10 (3%)	242 (81%)	3 (1%)	250 (80%)

[1]Hiatal hernia, distance >2 cm between diaphragmatic hiatus and the oesophagogastric junction;
[2]Oesophagitis, Savary-Miller grade I-IV;
[3]Reflux symptoms, heartburn and/or acid regurgitation > 50 times/year
Some of the total percentages in the tables do not equal 100 due to rounding off.
Analyses stratified by reflux symptoms and body mass index were only undertaken in 609 and 610 patients, respectively, for whom adequate questionnaire information was available.

9.4 Risk factor analysis (Paper I, III-IV)

9.4.1 Barrett's oesophagus

We included 604 consenting patients and 160 population controls (53% of the selected) with complete data in the analyses. The partly overlapping case groups are graphically represented by a Venn diagram in figure 6. Ninety-five patients exhibited macroscopic columnar metaplasia (MCM) above the OGJ (any length). Only 11 of them had a segment length >2 cm. Thirty-two had IM above the OGJ upon histological examination, and 21 of them also had MCM, thus meeting our criteria for Barrett's oesophagus. Eighteen of the latter had a visible segment length ≤2 cm. Among those with IM above the OGJ, 11 (6 with BO) also had IM below the OGJ. Another 4 with MCM not fulfilling our criteria for BO had such metaplasia. We regarded 498 patients with no MCM or IM above the OGJ as "endoscopy controls" (37 with IM below the OGJ).

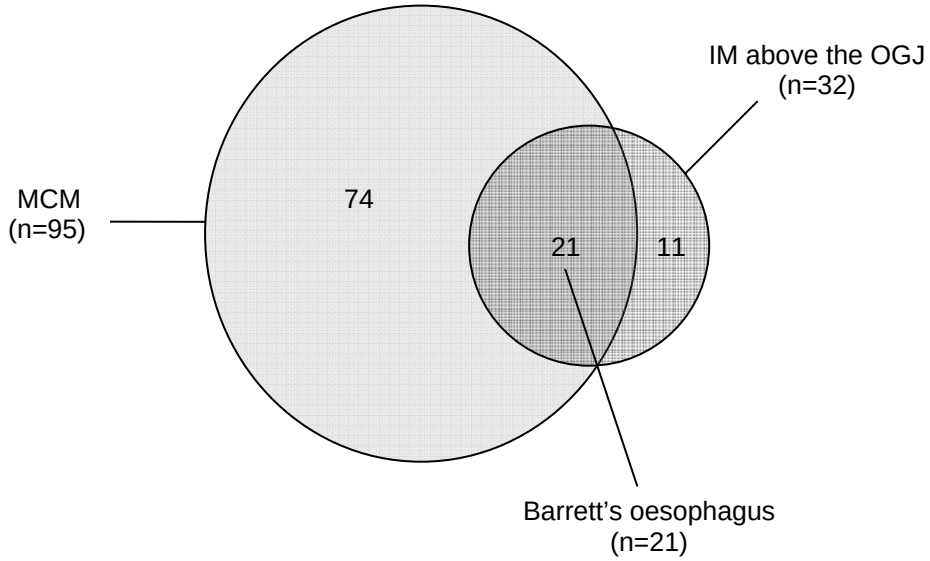

Figure 6: Numbers of patients in the three partly overlapping case groups. MCM = macroscopic columnar metaplasia above the oesophagogastric junction (OGJ); IM = histologically confirmed intestinal metaplasia. Twenty-one patients had both MCM and IM above the OGJ and were thus considered to have Barrett's oesophagus (the darkest area).

Demographics and clinical data of case and control groups are presented in Table 8. Manifestations of GORD were more common among patients with IM above the OGJ and/or MCM compared to endoscopy controls. The contrast was even greater when the case groups were compared with population controls. Average BMI did not differ substantially between the groups. Presence of *H. pylori* below the OGJ was equally common among patients with MCM and among the endoscopy controls, but more common among cases with IM above the OGJ and BO. The highest *H. pylori* prevalence (40%) was noted among the 52 cases and

Table 8. Demographics and clinical data of endoscopy patients (n=604) and population controls (n=160) included in the analyses.

Feature		Case groups			Control groups	
		MCM§ (n=95)	IM above the OGJ (n=32)	Barrett's oesophagus (n=21)	Endoscopy controls* (n=498)	Population controls¶ (n=160)
Age (yrs)	mean	56.0	61.0	60.3	51.4	61.8
	median	56	61	61	52	64
Gender (%)	men	42	34	29	43	34
	women	58	66	71	57	66
BMI, kg/m²(mean)		25.9	25.6	26.5	25.2	25.7
Symptoms of GORD (heartburn and/or acid regurgitation >50 times/ year)		45%	38%	48%	33%	10%
Proportion of endoscopies with suspicion of GORD /oesophagitis as main indication		52%	47%	43%	38%	-
Proportion of patients with hiatal hernia†		37%	25%	29%	21%	-
Proportion of patients with oesophagitis‡		33%	25%	29%	15%	-
Helicobacter pylori in gastric cardia biopsies		18%	31%	33%	18%	-
Tobacco smoking (ever smokers)		49%	50%	52%	41%	35%
Non-teetotallers		81%	69%	67%	83%	79%

†Hiatal hernia, distance >2 cm between diaphragmatic hiatus and the oesophagogastric junction
‡Oesophagitis, Savary-Miller grade I-IV (macroscopic columnar mucosa without other signs of oesofagitis not included, neither isolated Schatzki's ring)
*Endoscopy controls, patients with no evidence of intestinal metaplasia above the gastro-oesophageal junction and no macroscopic columnar metaplasia above the gastro-oesophageal junction
¶Not endoscoped, matched on age and sex to the patients with intestinal metaplasia above the gastro-oesophageal junction

controls with IM below the OGJ. In general smoking was reported more and alcohol consumption less often in the case groups than in the control groups.

In multivariable analyses (main effect models) confined to cases and endoscopy controls (Table 9) moderate positive associations (OR 1.5-2.0) emerged between GORD symptoms and MCM and/or IM above the OGJ, however statistically significant only for patients with MCM. Age was a significant risk factor, with a 5% (95% CI 1-9%) increase in BO prevalence per year. An 80% excess risk for BO among women, compared with men, did not reach statistical significance. The prevalence odds of MCM did not differ between sexes. BMI was essentially unrelated to presence of MCM or BO but tended to be inversely associated with IM above the OGJ. Ever-smoking, compared with never-smoking, was associated with a 40-80% excess prevalence of all three outcomes, albeit statistically non-significant. Alcohol use tended to be weakly negatively related to BO and IM above the OGJ. While subjunctional presence of *H. pylori* was linked to a non-significant 70% elevation in the odds of having BO, the association with MCM tended to be inverse; thus, the overall relationship between subjunctional *H. pylori* and the three variants of metaplasia in the oesophagus was unimpressive.

The results of similarly multi-adjusted logistic regression models based on the comparison between case patients and population controls (Table 10) unveiled associations that all went in the same direction as those observed in the comparison with endoscopy controls, but the reflux–metaplasia and smoking–metaplasia relationships were much stronger. The OR for BO among individuals with reflux symptoms, relative to those without, was 10.7 (95% CI 3.5-33.4); among ever-smokers, relative to never-smokers, it was 3.3 (95% CI 1.1-9.9). As in the comparison with endoscopy controls, there was no consistent relationship between BMI and the three outcomes, and alcohol use remained inversely, but non-significantly, associated with the odds of having either of the three types of oesophageal metaplasia. Effects of age and gender could not be evaluated due to the frequency matching on these variables, and *H. pylori* was not measured in the population controls.

Associations with long segment (≥ 3cm) BO could not be evaluated due to low numbers. However, 8 patients, all women, had endoscopically visible columnar epithelium above the OGJ of ≥ 2 cm, confirmed by intestinal metaplasia at biopsy. Multivariable analyses including these 8 cases and the endoscopy controls were undertaken (main effect model, restriction made to women, BMI dichotomized – there were no cases in the middle BMI tertile). Significant associations emerged with age and reflux symptoms (OR=1.11, 95% CI 1.02-1.21 and OR=7.3, 95% CI 1.2-46.1, respectively). There were also still positive, although non-significant, associations with *H. pylori* and smoking (OR=3.1, 95% CI 0.6-17.5 and OR=1.8, 95% CI 0.3-10.0, respectively). The inverse association with alcohol use attained significance (OR=0.2, 95% CI 0.0-0.9) and high (>24.4) BMI tended to be inversely associated with BO ≥2 cm (OR=0.4, 95% CI 0.1-2.2). Similar analyses based on the comparison between case patients and population controls revealed associations with reflux symptoms, smoking, alcohol use and BMI that all went in the same direction as those observed in the comparison with endoscopy controls (age and *H. pylori* not applicable), significant for reflux symptoms and alcohol use (data not shown).

Table 9. Relationships* between selected background factors and probability of having macroscopic columnar metaplasia above the OGJ (MCM), intestinal metaplasia (IM) above the oesophagogastric junction (OGJ), or both, thus fulfilling the diagnostic criteria of Barrett's oesophagus (BO). Reference group in each comparison was endoscopy patients without MCM or IM above the OGJ (n=498).

Factor	MCM (95 cases, 498 controls)		IM above the OGJ (32 cases, 498 controls)		Barrett's oesophagus (21 cases, 498 controls)	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
Age (continuous)	1.03	(1.01-1.05)	1.05	(1.02-1.09)	1.05	(1.01-1.09)
Sex						
Male	1.0	(ref)	1.0	(ref)	1.0	(ref)
Female	1.0	(0.7-1.7)	1.4	(0.6-3.1)	1.8	(0.7-5.2)
Reflux symptoms						
≤50 times/year	1.0	(ref)	1.0	(ref)	1.0	(ref)
>50 times/year	1.7	(1.1-2.8)	1.5	(0.7-3.2)	2.0	(0.8-5.0)
Body mass index (BMI, kg/m²)						
Low tertile (<23.6)	1.0	(ref)	1.0	(ref)	1.0	(ref)
Middle tertile (23.6-26.6)	1.0	(0.5-1.7)	0.9	(0.4-2.1)	0.9	(0.3-2.9)
High tertile (>26.6)	1.2	(0.7-2.1)	0.7	(0.3-1.8)	1.1	(0.3-3.3)
Helicobacter pylori						
Negative	1.0	(ref)	1.0	(ref)	1.0	(ref)
Positive	0.8	(0.5-1.5)	1.5	(0.7-3.5)	1.7	(0.7-4.6)
Smoking						
Never	1.0	(ref)	1.0	(ref)	1.0	(ref)
Ever	1.4	(0.9-2.3)	1.5	(0.7-3.3)	1.8	(0.7-4.4)
Alcohol consumption						
Abstainer	1.0	(ref)	1.0	(ref)	1.0	(ref)
User	1.0	(0.5-1.8)	0.7	(0.3-1.5)	0.6	(0.2-1.7)

*Results obtained from multivariable logistic regression, with mutual adjustments for all factors in the table.

Table 10. Relationships* between selected background factors and probability of having macroscopic columnar metaplasia above the OGJ (MCM), intestinal metaplasia (IM) above the OGJ, or both, thus fulfilling the diagnostic criteria of Barrett's oesophagus (BO). Reference group in each comparison was 160 population controls (not endoscoped), matched to patients with IM above the OGJ on age and sex.

Factor	MCM (95 cases, 160 controls)		IM above the OGJ (32 cases, 160 controls)		Barrett's oesophagus (21 cases, 160 controls)	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
Reflux symptoms						
≤50 times/year	1.0	(ref)	1.0	(ref)	1.0	(ref)
>50 times/year	7.4	(3.7-14.9)	7.1	(2.7-19.1)	10.7	(3.5-33.4)
Body mass index (BMI, kg/m²)						
Low tertile (<23.6)	1.0	(ref)	1.0	(ref)	1.0	(ref)
Middle tertile (23.6-26.6)	1.3	(0.6-2.7)	1.2	(0.4-3.4)	1.9	(0.5-7.4)
High tertile (>26.6)	1.2	(0.6-2.5)	0.6	(0.2-1.7)	1.2	(0.3-4.5)
Smoking						
Never	1.0	(ref)	1.0	(ref)	1.0	(ref)
Ever	1.8	(1.0-3.3)	2.8	(1.1-6.9)	3.3	(1.1-9.9)
Alcohol consumption						
Abstainer	1.0	(ref)	1.0	(ref)	1.0	(ref)
User	0.6	(0.3-1.3)	0.4	(0.2-1.2)	0.4	(0.1-1.4)

*Results obtained from multivariable logistic regression, with mutual adjustments for all factors in the table together with the matching factors (sex and age).

9.4.1.1 Evaluation of potential effect modification

In order to evaluate possible effect modification, we estimated ORs for oesophageal metaplasia in the four possible permutations of reflux and *H. pylori* (–/–, +/–, –/+ and +/+) with –/– being the reference category (Table 11). This analysis could only be done using endoscopy controls. Symptomatic reflux, in the absence of *H. pylori*, was borderline associated with MCM but not with IM above the OGJ or BO. *H. pylori*, in the absence of reflux symptoms, tended to be inversely associated with MCM and BO, but not with IM above the OGJ. The combination of reflux symptoms and presence of *H. pylori* in the cardia, on the other hand, was positively, strongly and significantly associated with BO (OR=4.8, 95% CI 1.4-16.5) and borderline significantly associated with IM above the OGJ (OR=3.2, 95% CI 1.0-10.3), but not with MCM (OR=1.4, 95% CI 0.6-3.5). The interaction between reflux and *H. pylori* did not quite reach statistical significance for any of the outcomes (p=0.072, p=0.055 and p=0.99 for BO, IM above the OGJ and MCM, respectively). Tests for effect modification of the reflux–metaplasia relationships by BMI, performed both with endoscopy controls and population controls, did not reveal any important interactions (data not shown). Insufficient number of cases in the different categories did not allow meaningful effect modification evaluation restricted to cases with BO segments ≥ 2 cm.

Table 11. The combined effect of reflux and subjunctional presence of *Helicobacter pylori**. Reference group (n=498) in each comparison was endoscopy patients without macroscopic columnar metaplasia (MCM) or histological intestinal metaplasia (IM) above the oesophagogastric junction (OGJ).

		MCM (95 cases, 498 controls)			IM above the OGJ (32 cases, 498 controls)			Barrett's oesophagus (21 cases, 498 controls)		
Reflux symptoms	*H. pylori*	(cases/ controls)	OR	(95% CI)	(cases/ controls)	OR	(95% CI)	(cases/ controls)	OR	(95% CI)
-	-	(42/272)	1.0	(ref)	(15/272)	1.0	(ref)	(9/272)	1.0	(ref)
+	-	(36/134)	1.7	(1.0-2.9)	(7/134)	1.0	(0.4-2.7)	(5/134)	1.1	(0.4-3.5)
-	+	(10/64)	0.8	(0.4-1.7)	(5/64)	1.0	(0.3-2.8)	(2/64)	0.7	(0.1-3.3)
+	+	(7/28)	1.4	(0.6-3.5)	(5/28)	3.2	(1.0-10.3)	(5/28)	4.8	(1.4-16.5)

*Results obtained from multivariable logistic regression. Other variables included in the models were age (continuous), sex, body mass index (three categories), tobacco smoking (ever/never) and alcohol consumption (user/abstainer).
Tests for multiplicative interaction (reflux symptoms**Helicobacter pylori*, likelihood ratio test) did not reach statistical significance (p<0.05) for any of the outcomes.

9.4.2 Cardiac mucosa in the gastric cardia

With mutual adjustments (including age and gender) in a logistic regression model neither reflux symptoms, nor BMI, nor smoking nor histologic evidence of *H. pylori* colonization in the proximal stomach was significantly associated with
the presence of cardiac mucosa in the cardia (data not shown).

9.4.3 Pancreatic acinar metaplasia

Our notion that presence of PAM seemed to be restricted to either side of the OGJ prompted us to analyze the patients with PAM not only as a whole, but also divided into patients with PAM above and patients with PAM below the OGJ. In Table 12 demographics and clinical data of 604 consecutive patients and 160 population controls with complete data are presented. The mean age in patients with PAM in any biopsy was slightly higher than in patients without PAM. In patients with PAM above the OGJ, with or without PAM below the OGJ, there was a female predominance exceeding that among the endoscopy controls. Manifestations of GORD were, overall, more common among patients with PAM above the OGJ than among patients with PAM below the OGJ, endoscopy controls and population controls. Moreover subjunctional presence of *Helicobacter pylori* was noted considerably more often in patients with PAM above the OGJ than in the other endoscopy patients. Average BMI and alcohol consumption did not differ substantially between the groups, while smoking was reported more often among the endoscopy patients compared to the population controls, and seemed to be most common among patients with PAM above the OGJ.

In multivariable analyses confined to endoscopy patients (Table 13) we found PAM to be weakly and borderline significantly associated with age in all case groups. PAM below the OGJ was inversely associated with female gender (OR 0.6, 95% CI 0.4-1.0), while PAM above the OGJ showed moderate, non-significant, positive associations with female gender (OR 1.8, 95% CI 0.9-3.3) and reflux symptoms (OR 1.7, 95% CI 0.9-3.1). A stronger, albeit still borderline significant, association was seen between PAM above the OGJ and subjunctional presence of *Helicobacter pylori* (OR 2.0, 95% CI 1.0-4.0). There were no consistent associations between BMI, smoking and alcohol consumption with regard to the investigated outcomes.

In order to further explore the associations with PAM above the OGJ and to minimize the influence of PAM below the OGJ, we also analyzed patients with PAM only above the OGJ, excluding case patients with PAM both above and below the OGJ. The results of these analyses (Table 13, last column) further supported correlations described above. The ORs associated with female gender and *Helicobacter pylori* presence increased and were statistically significant. The link to reflux symptoms also became slightly stronger, however still not statistically significant.

Table 14 presents the results of the multivariable analyses that included case patients with PAM and population controls. Reflux symptoms was significantly associated with all the investigated outcomes, but in accordance with the analyses restricted to endoscopy patients PAM above the OGJ, and in particular PAM only above the OGJ, were more strongly linked to such symptoms (OR 7.6, 95% CI 3.3-17.3 and OR 9.5, 95% CI 3.8-23.6, respectively) than was PAM below the OGJ and PAM overall. No significant associations were seen for the other investigated factors BMI, smoking and alcohol consumption, well in line with the analyses confined to endoscopy patients. *H. pylori* was not measured in the population controls and effects of age and gender could not be evaluated due to the frequency matching.

Table 12. Demographics and clinical data of endoscopy patients (n=604) and population controls (n=160) included in the multivariable analyses.

Feature		Case groups			Control groups	
		PAM below and/or above OGJ (n=116)	PAM below OGJ (n=77)	PAM above OGJ (n=50)	Endoscopy controls‡ (n=488)	Population controls§ (n=160)
Age (yrs)	mean	55.1	54.8	54.6	51.6	61.8
	median	56	57	57	52	64
Gender (%)	men	43	53	32	43	34
	women	57	47	68	57	66
BMI, kg/m²(mean)		25.2	25.1	25.4	25.3	25.7
Symptoms of GORD (heartburn and/or acid regurgitation >50 times/ year)		38%	32%	46%	33%	10%
Proportion of endoscopies with suspicion of GORD /oesophagitis as main indication		38%	36%	38%	41%	-
Proportion of patients with hiatal hernia*		19%	17%	24%	24%	-
Proportion of patients with oesophagitis†		16%	12%	22%	19%	-
Helicobacter pylori in gastric cardia biopsies		23%	17%	30%	17%	-
Tobacco smoking (ever smokers)		41%	38%	48%	42%	35%
Non-teetotallers		81%	81%	84%	82%	79%

*Hiatal hernia, distance >2 cm between diaphragmatic hiatus and the gastro-oesophageal junction
†Oesophagitis, Savary-Miller grade I-IV (macroscopic columnar mucosa without other signs of oesofagitis not included, neither isolated Schatzki's ring)
‡Patients with no evidence of PAM in any biopsy
§Not endoscoped, matched on age and sex to the patients with intestinal metaplasia above the gastro-oesophageal junction

Table 13. Relationships* between selected background factors and probability of having pancreatic acinar metaplasia (PAM) below or above the gastro-oesophageal junction (GOJ). Reference group in each comparison was patients without PAM in any biopsy (n=488).

Factor	PAM below and/or above OGJ (116 cases, 488 controls)		PAM below OGJ (77 cases, 488 controls)		PAM above OGJ (50 cases, 488 controls)		PAM only above OGJ (39 cases, 488 controls)	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
Age (continuous)	1.02	(1.00-1.03)	1.02	(1.00-1.04)	1.01	(0.99-1.04)	1.02	(1.00-1.05)
Sex								
Male	1.0	(ref)	1.0	(ref)	1.0	(ref)	1.0	(ref)
Female	1.0	(0.6-1.5)	0.6	(0.4-1.0)	1.8	(0.9-3.3)	2.8	(1.3-6.3)
Reflux symptoms								
≤50 times/year	1.0	(ref)	1.0	(ref)	1.0	(ref)	1.0	(ref)
>50 times/year	1.3	(0.8-2.0)	1.1	(0.6-1.8)	1.7	(0.9-3.1)	1.9	(0.9-3.8)
Body mass index (BMI, kg/m²)								
Low tertile (<23.6)	1.0	(ref)	1.0	(ref)	1.0	(ref)	1.0	(ref)
Middle tertile (23.6-26.6)	1.2	(0.7-2.0)	1.0	(0.5-1.8)	1.6	(0.7-3.4)	1.5	(0.6-3.5)
High tertile (>26.6)	0.9	(0.5-1.5)	0.7	(0.4-1.4)	1.0	(0.5-2.3)	1.2	(0.5-2.9)
Helicobacter pylori[§]								
Negative	1.0	(ref)	1.0	(ref)	1.0	(ref)	1.0	(ref)
Positive	1.3	(0.8-2.2)	0.8	(0.4-1.6)	2.0	(1.0-4.0)	2.6	(1.3-5.4)
Smoking								
Never	1.0	(ref)	1.0	(ref)	1.0	(ref)	1.0	(ref)
Ever	0.9	(0.6-1.4)	0.8	(0.5-1.3)	1.2	(0.6-2.1)	1.2	(0.6-2.5)
Alcohol consumption								
Abstainer	1.0	(ref)	1.0	(ref)	1.0	(ref)	1.0	(ref)
User	1.1	(0.6-1.9)	0.9	(0.5-1.8)	1.4	(0.6-3.2)	1.4	(0.5-3.4)

*Results obtained from multivariable logistic regression, with mutual adjustments for all factors in the table.

[§]Based on histology from the subjunctional area.

Table 14. Relationships* between selected background factors and probability of having pancreatic acinar metaplasia (PAM) below or above the oesophagogastric junction (OGJ). Reference group in each comparison was 160 population controls (not endoscoped), matched to patients with IM above the OGJ on age and sex.

Factor	PAM below and/or above OGJ (116 cases, 160 controls)		PAM below OGJ (77 cases, 160 controls)		PAM above OGJ (50 cases, 160 controls		PAM only above OGJ (39 cases, 160 controls	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
Reflux symptoms								
≤50 times/year	1.0	(ref)	1.0	(ref)	1.0	(ref)	1.0	(ref)
>50 times/year	5.8	(2.9-11.3)	4.5	(2.1-9.8)	7.6	(3.3-17.3)	9.5	(3.8-23.6)
Body mass index (BMI, kg/m²)								
Low tertile (<23.6)	1.0	(ref)	1.0	(ref)	1.0	(ref)	1.0	(ref)
Middle tertile (23.6-26.6)	1.4	(0.7-2.6)	1.2	(0.6-2.5)	2.5	(1.0-6.5)	2.2	(0.8-6.2)
High tertile (>26.6)	1.0	(0.5-2.0)	0.8	(0.4-1.8)	1.4	(0.5-3.8)	1.4	(0.5-4.1)
Smoking								
Never	1.0	(ref)	1.0	(ref)	1.0	(ref)	1.0	(ref)
Ever	1.3	(0.7-2.2)	1.2	(0.6-2.2)	1.5	(0.7-3.1)	1.5	(0.6-3.6)
Alcohol consumption								
Abstainer	1.0	(ref)	1.0	(ref)	1.0	(ref)	1.0	(ref)
User	0.7	(0.4-1.5)	0.7	(0.3-1.6)	0.8	(0.3-2.3)	0.8	(0.2-2.3)

*Results obtained from multivariable logistic regression, with mutual adjustments for all factors in the table together with the matching factors (sex and age).

9.4.3.1 Correlation with acid reflux and Barrett's oesophagus (24 hour pH metry)

We further invited 49 of the study patients (with questionnaire information on reflux symptoms and still alive) from one centre (Kalmar) to a supplementary 24 hour oesophageal pH monitoring: 30 randomly selected patients (out of 42 eligible) with PAM above the OGJ and no Barrett's oesophagus, all 7 patients with Barrett's oesophagus *and* PAM above the OGJ and all 12 patients with Barrett's oesophagus *without* PAM above the OGJ. Twenty-six patients (53%) accepted, and the results from the pH monitoring together with clinical data of the patients are presented in Table 15. Due to low numbers comparisons between the three groups were of low statistical power, but the mean values of percent of total time with oesophageal pH<4, percent of supine time with pH<4 and percent of upright time with pH<4 were not substantially different across the three groups. However, in relation to the reference for the method as regards percent of total time with pH<4 (3.4%) the results among PAM patients with and without accompanying BO (7.7%, 95% CI 0.3-15.1% and 7.1%, 95% CI 3.4-10.8%, respectively), suggest that abnormal reflux patterns may be associated with the development of PAM. Percent of total time pH<4 for the individual patients are presented figure 7.

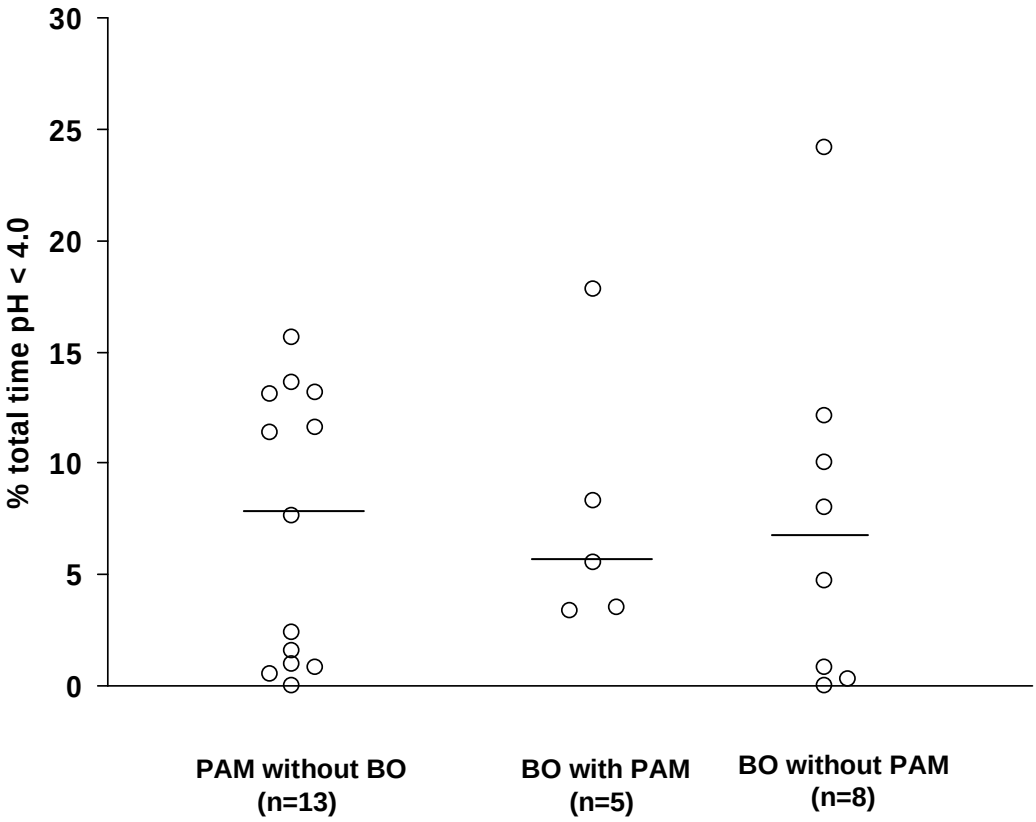

Figure 7. Percentage of total time with oesophageal pH<4 for the individual 26 patients with the three combinations of presence/absence of pancreatic acinar metaplasia (PAM) above the oesophagogastric junction and Barrett's oesophagus (BO). The horizontal lines denote the median of each group.

Table 15. Patient characteristics and results of the 24 hour pH monitoring (n=26).

	PAM without BO (n=13)	BO with PAM (n=5)	BO without PAM (n=8)
Age (yrs)			
mean	51.6	60.8	56.6
median	55	61	58
Gender			
men/ women (%)	38/62	0/100	50/50
Distribution of short (SS) and long (LS) segment BO	- -	SSBO: 4 LSBO: 1	SSBO: 6 LSBO: 2
Reflux symptoms	46% (6/13)	60% (3/5)	50% (4/8)
Percent of total time pH<4 (ref. 3.4%)			
mean	7.1	7.7	7.5
median	7.6	5.5	6.4
range	0-15.6	3.4-17.8	0–24.2
95% CI	3.4-10.8	0.3-15.1	0.7-14.3
Percent of supine time pH<4 (ref. 1.2%)			
mean	3.0	3.7	5.5
median	0.1	1.5	0.6
range	0-14.7	0-9.2	0-18.9
Percent of upright time pH<4 (ref. 6.3%)			
mean	9.1	9.8	8.7
median	9.8	7.6	7.8
range	0-20.9	4.5-22.6	0-29.4
Proportion pathological investigations (>3.4% of the total time pH<4)	54% (7/13)	80% (4/5)	62% (5/8)
Symptom index (SI)[2] % (numbers reporting symptoms during pH monitoring)	(7=54%)	(3=60%)	(1=12%)
mean	93	61	100
median	100	83	100
range	71-100	0-100	100-100

[2]Symptom index (SI), (number of symptoms correlated to a pH value <4 / total number of symptoms) x 100

10 GENERAL DISCUSSION

10.1 Methodological considerations

10.1.1 Study design

One of our goals was to estimate the prevalence of Barrett's oesophagus in the Swedish population, but since endoscopies in healthy people are ethically problematic and associated with considerable risk of selection bias, we investigated all patients coming for a first-time endoscopy from a geographically defined population. Hence, the study was designed to come close to a truly population-based epidemiological evaluation, with its anchorage in defined catchment area populations.

10.1.2 Selection bias

With regard to prevalence studies one source of bias is the selection of cases that will be objects of the diagnostic procedure. If the selection process is influenced by factors affecting the occurrence of the condition under study this will lead to a distorted estimation of the true prevalence. The participation rate in our study was estimated at 788/1361 (58%). Since Barrett's oesophagus has a strong known correlation with gastro-oesophageal reflux a potentially important difference between participating and non-participating patients was a higher proportion investigated for reflux among the former. However, when we re-calculated the prevalence figures stratified for indication our overall estimates of IM and Barrett's oesophagus prevalence were barely affected since the prevalence was similar in those investigated because of reflux and those investigated for other reasons.

Selection bias can occur whenever individual subjects for inclusion into the study are selected as cases, or controls, due to their association with the exposure(s). Strengths of our study include our attempts to reduce selection bias: We opted to investigate all individuals coming for endoscopy, regardless of symptoms, during a defined time period in a well-defined catchment area population. We restricted the study to people with a new indication for their endoscopy, and avoided the inclusion of a selected group of "known" prevalent cases under surveillance. Although the controls came from the same population as the columnar metaplasia cases, some caution is warranted; endoscopy patients are not truly representative of an underlying source population. They are likely to be more exposed to risk factors for gastrointestinal symptoms, including smoking and *H. pylori* infection. They might also be subject to selection forces that are related to doctors' perceptions of indications for endoscopy. Age, sex, and smoking status may be factors that influence the referral decision. With higher exposure prevalence to these factors among the endoscopy controls than in the true source population that generated the columnar metaplasia cases, relative risk estimates tend to be biased towards lower values. On the other hand, also the metaplasia cases may be a similarly selected substratum of the entire prevalence pool of cases in the source population. Hence, in the comparison with population controls, relative risk estimates might have been biased upwards for some factors related to the probability of seeing a doctor (like severe symptoms) or being referred for endoscopy (like smoking). The true strength of the association for these factors may, therefore, fall somewhere between the values observed in the comparison with endoscopy controls and those obtained in the comparison with population controls.

With regard to the population controls the prevalence of reflux symptoms was 8% and 16% among quick and reluctant responders, respectively. Assuming that the latter were more similar to the non-participants it appears that the non-participation might have led to some

underestimation of the prevalence of reflux symptoms in the population controls. Together with the tendency towards selection of cases with reflux symptoms, the association between such symptoms and BO may have been overestimated. We observed no important differences between quick and reluctant responders with regard to BMI, smoking prevalence, or proportion alcohol abstainers.

10.1.3 Misclassification

If the study subjects are incorrectly classified with regard to the condition under study the conclusions regarding its prevalence will of course be unreliable. Biopsies were taken immediately above and below the OGJ but not exactly at the squamocolumnar junction and therefore we might have missed some cases of the different types of metaplasia, although two biopsies were obtained at each site.

Our finding of columnar mucosa above the OGJ in 27% of the patients with no apparent macroscopic columnar-lined oesophagus might raise concerns about a general problem with obtaining biopsies from correct locations, involving risk of misclassification bias.

With regard to PAM differential misclassification of location in relation to subjunctional *H. pylori* status or sex seems improbable, but such misclassification in relation to presence or absence of reflux symptoms cannot be excluded because of difficulties in orientation associated with GORD-related hiatal hernia. However, all endoscopies were performed (or supervised) by experienced endoscopists and in accordance with a protocol where the anatomical landmarks were emphasized. A special concern regarding the importance of reflux symptoms is, as demonstrated in our previous report (Johansson et al. 2005), that participating endoscopy patients tended to be investigated for reflux symptoms more often than were non-participants, and that non-participation appeared to have led to some underestimation of the prevalence of reflux symptoms in the population controls (Johansson et al.). It is, however, inconceivable that these over- and underrepresentations of individuals with reflux symptoms would be conditional on the covert presence or absence of PAM. Therefore, it is unlikely that the associations between reflux and PAM would be seriously affected.

Factors related to diagnostic accuracy of columnar metaplasia and Barrett's oesophagus are specifically studied and discussed below.

The population controls were not endoscoped and most probably some members of this control group had BO (or PAM) that remained undiagnosed. However, according to a recent population-based study by Ronkainen et al (Ronkainen et al. 2005) this potential misclassification of BO would be in the order of 1-2% and assumingly ignorable with regard to our analyses.

10.1.4 Chance

In the case of an observed association chance needs to be considered as an alternative explanation. Ways of evaluating chance are significance testing and confidence interval estimation.

The small sample size resulted in a limited number of observed metaplasia cases, with unstable point estimates (wide confidence intervals) and a high probability of type II errors as obvious consequences (i.e. inability to find true associations). Except for the effect of age, none of the associations with BO (overall) were statistically significant in the analysis with endoscopy controls (although the subgroup analysis of BO ≥2 cm, which were all women,

revealed significant associations also with reflux symptoms and, inverse, with alcohol consumption). Consistent, stronger, and statistically significant results in the comparison with population controls, however, made our data more interpretable.

Also regarding associations with measured acid reflux the precision in our data was very limited owing to that, for ethical and economical reasons, only a small sample of volunteering patients underwent oesophageal pH monitoring.

10.2 Findings and implications

10.2.1 Prevalence of Barrett's oesophagus and intestinal metaplasia

Endoscopic long-segment (≥3 cm) columnar epithelium in the distal oesophagus was noted in 3% of the patients, and short segment ditto was seen in 14%; 14% had histologic intestinal metaplasia (IM) immediately below or above the OGJ. Long and short segment columnar epithelium with histologically confirmed IM was recorded in 0.8% and 3%, respectively. Pancreatic acinar metaplasia was observed in 18%, with no significant relationship with age, sex, reflux symptoms, BMI or presence of *H. pylori.*

Our prevalence figures are in the lower range compared to previous studies of gastroscopy patients, except for LSBO, which was reported in around 1% in most studies (Cameron 1997; Hirota et al. 1999). One explanation might be that we only took biopsies slightly above and below the OGJ, and no more than two biopsies in each location. It appears unlikely, however, that the prevalence of IM would peak to the extent in the few millimeters of OGJ not covered by our tissue sampling that our observed prevalence figures would change dramatically. One of the larger previous studies (Hirota et al. 1999) reported the same overall IM prevalence (13.2%) among American patients as in our study, a lower prevalence of IM-OGJ (5.6%), but a higher prevalence of SSBO (6.0%). The differences regarding IM-OGJ and SSBO might to some extent be related to misclassification of the biopsy level in our study, where photo documentation was not required. Another large Finnish study (Voutilainen et al. 1999a) reported junctional IM (complete and/or incomplete) in 22%. The explanation for this rather high figure might be sought in the prevalence of suggested pathogenetic factors. For example, cardial *Helicobacter pylori* infections were more prevalent in the Finnish population (26%) than in our (18%) and in the American one (8.8%). Further the Finnish patients were slightly older than both the American and our patients. A recent Swedish study, however, puts our figures in a better perspective. In a random sample of the adult population they found Barrett's oesophagus in only 1.6% (Ronkainen et al. 2005); short segment BO were found in 1.1% and long segments in 0.5%.

The female predominance among IM and IM-OGJ patients in our study is consistent with the above-mentioned American and Finnish results.

BO and IM in the OGJ region are widely accepted as acquired lesions albeit with possibly different aetiologies (Cameron 1997; Voutilainen et al. 1999a; Glickman et al. 2001). The age trends in IM overall and IM-OGJ in our study, and the higher mean ages among patients with IM overall, IM-GOJ, SSBO, LSBO compared to the whole study population, support the acquired nature of these lesions. Another, however less likely, explanation of the age trends is a decrease in prevalence across successive birth cohorts.

10.2.2 Cardia type mucosa in the gastric cardia

Our finding of only gastric-fundic (i.e. no cardiac type) mucosa immediately below the OGJ in 56% of the patients are consistent with those of two autopsy series where "pure cardiac mucosa" was absent in 29% and 56% respectively (Chandrasoma et al. 2000a). Also in these series, there was a tendency for the presence and the extent of cardiac mucosa to increase with age. Taken together, these data are consistent with the hypothesis that cardiac mucosa might be acquired – or at least expanded during adult life. An alternative, albeit less plausible, explanation is that the presence of cardiac mucosa is a birth cohort phenomenon. A recent study among paediatric autopsy cases (Kilgore et al. 2000), however, contradicts this notion: cardiac mucosa was observed on the gastric side of the OGJ in every case although the length of the cardiac mucosa could be as little as 1.0 mm. But these results do not exclude the possibility of an age-dependent expansion of this region.

10.2.3 Diagnostic accuracy

Misclassification of Barrett's oesophagus can occur for several reasons. First, the macroscopic evaluation can be wrong, e.g., normal squamous epithelium is confused with columnar, or normal subjunctional columnar mucosa is believed to be located above the oesophagogastric junction. Second, the pathologist may misinterpret the histological picture. Third, the endoscopist may fail to position the biopsy forceps in the intended area and instead take biopsies below the oesophagogastric junction or above the metaplastic segment. Therefore, when interpreting discordant results from the macro- and microscopic evaluations, there is no solid gold standard – either or both could be wrong. Moreover, the agreement is expected to be less if the endoscopic evaluation is to be compared with histologically confirmed intestinal metaplasia (which is a subgroup among all cases with columnar epithelium above the oesophagogastric junction) than if just any columnar epithelium serves as the histologic standard. In the former case, an endoscopic finding of columnar epithelium and a histologic finding of metaplastic columnar mucosa of cardiac or gastric-fundic type will be classified as discrepant, while in the latter case this combination will be concordant.

Regardless of whether intestinal metaplasia or just any columnar epithelium served as histologic standard, the concordance between the macrocopic and microscopic evaluations were far from perfect in our study; the overall concordance rate with any columnar epithelium as the histologic standard was 74%, and the K value (0.38) indicated only fair (Altman 1991) agreement beyond chance. Contrary to the naïve expectation we noted a higher concordance rate (86%) when intestinal metaplasia was the histologic standard. This was because the low prevalence of intestinal metaplasia placed the overwhelming majority of evaluated patients in the "no intestinal metaplasia" category upon histological examination. As the great majority of all patients were also deemed to lack macroscopic signs of columnar mucosa in the oesophagus at endoscopy, no less than 82% of all patients fell into the concordant category "no macroscopic columnar mucosa and no histologic intestinal metaplasia". As expected, K was somewhat lower (0.31) than when any columnar epithelium in the oesophagus served as the histologic standard.

The macroscopic evaluation at endoscopy "missed" more than half of the cases with histologically confirmed columnar mucosa of any kind above the oesophagogastric junction. On the other hand, the number of "false positive" cases, i.e., patients classified as having columnar mucosa based on the macroscopic evaluation but with no evidence of such metaplasia in the histological sections, was small. Hence, the positive predictive value of a macroscopic finding vis-à-vis microscopic presence of columnar epithelium was high (86%) in this patient population, while the corresponding negative predictive value (72%) was less

impressive. An alternative explanation would be that some or most of the "false negative" cases had correct macroscopic diagnoses but erroneous histological evaluations, for instance due to biopsies that were incorrectly targeted below the oesophagogastric junction. In our study, specialized intestinal metaplasia constituted no more than 16% of all allegedly metaplastic columnar epithelium, and this raises the question if all of the findings of cardiac and gastric-fundic mucosa were really made above the oesophagogastric junction. Erroneously targeted biopsies, however, constitute a less likely explanation since the prevalence of histologic columnar mucosa remained unchanged among patients with a hiatal hernia (where the risk of inadvertent subjunctional biopsies is greater) compared to patients without. Yet, the endoscopic "sensitivity" vis-à-vis histologic columnar mucosa increased, i.e., the "true positive" rate increased at the expense of the "false negative" rate. This suggests that the presence of a hiatal hernia (or similarly, a history of reflux symptoms, higher age, or presence of manifest oesophagitis) made the endoscopists more aware of the possibility of Barrett's oesophagus and increased their attention towards subtle signs of columnar mucosa in the oesophagus. In turn, this suggests that attempts to reduce misclassification of columnar mucosa above the oesophagogastric junction should be directed more to endoscopists than to pathologists. It must be emphasized that our data were obtained within the confines of a study. Thus, the attention towards columnar metaplasia was already increased, and the protocol advised the endoscopists regarding anatomical landmarks as well as proper sites for biopsies. In routine care, misclassification of oesophageal columnar metaplasia may be more common.

While the presumed greater attention among the endoscopists to the possibility of columnar metaplasia increased the agreement in patients with hiatal hernia and/or oesophagitis when any columnar metaplasia served as the histologic standard, the agreement tended to be lower – seemingly paradoxically – in these patients when intestinal metaplasia was the histologic standard. As expected, "false positive" macroscopic evaluations were more frequent, and the endoscopists' increased awareness in patients with hiatal hernia and/or oesophagitis may have added further to this rate. It could be argued that sampling error in biopsies from small tongues of Barrett's oesophagus in conjunction with oesphagitis would be an alternative explanation why the macroscopic "false positive" rate increased, i.e., that the histologic standard was wrong. Clearly, short segment lesions were in the majority among our patients (Johansson et al. 2005), and the yield of intestinal metaplasia is expected to be less from such lesions (Padda and Ramirez 2001; Öberg et al. 2001). However, the prevalence of histologically confirmed intestinal metaplasia increased more in patients with oesophagitis than in those with hiatal hernia, where this sampling problem might have been less critical. Moreover, in the 115 patients with endoscopically visible columnar mucosa above the oesophagogastric junction an average of 3.9 biopsies were obtained (median: 4, range 2-8), which is in accordance with "a range of good and presumably representative centres" in the United Kingdom, where the mean number of biopsies taken at diagnostic endoscopies was 3.8 (Coad and Shepherd 2003). Nevertheless in 61 patients the number of biopsies was not strictly according to protocol, which might have lowered the probability of ascertaining columnar mucosa (including intestinal metaplasia) in the distal oesophagus.

Most previous studies of the agreement between histology and macroscopic evaluation upon endoscopy have been confined to patients in whom Barrett's oesophagus was particularly suspected. In the present study we address this agreement in unselected consecutive new patients coming for endoscopy, regardless of indication. In this setting we expected that the sensitivity of the macroscopic evaluation vis-à-vis histology would be lower and the specificity would be higher, i.e., the "false negative" rate would be higher and the "false positive" rate would be lower, compared to investigations of accuracy in patients with an *a*

priori suspicion of Barrett's oesophagus. Notwithstanding, the overall concordance and agreement beyond chance was surprisingly similar. In an American study among patients with symptoms of gastro-oesophageal reflux disease (Dibble et al. 2004), the overall concordance with any histological diagnosis of Barrett's oesophagus (with type of metaplasia undefined) was 81% and the K value 0.43. In the latter study the comparisons were done only in patients who had oesophageal biopsies taken for clinical reasons. Suspicion of Barrett's oesophagus is one of the major clinical reasons for taking such biopsies. Woolf et al (Woolf et al. 1989) reported a concordance of no less than 91% (21/23), but they used the traditional ≥3 cm endoscopic criteria for the Barrett diagnosis, avoiding the difficulties associated with endoscopic classification of short segment columnar mucosa. Furthermore, they used Lugol's stain in the oesophagus during the endoscopic procedure. Among studies with intestinal metaplasia as the histologic standard, Eloubeidi and Provenzale (Eloubeidi and Provenzale 1999) found an overall concordance of 81% (corresponding K 0.39), but the comparisons were again confined to the subset of patients who had biopsies taken on clinical indications. However, one recent study prospectively analyzed unselected endoscopy patients for the endoscopic and histological (intestinal metaplasia type II or III) prevalence of Barrett's oesophagus, but also with a similar concordance rate of 81% (corresponding K value 0.39) (Endlicher et al. 2005a).

The relatively high frequency "false negative" endoscopic assessments, i.e., histologically confirmed intestinal metaplasia immediately above a macroscopically normal-appearing oesophagogastric junction, is in line with reports from several centres (Spechler et al. 1994a; Oberg et al. 1997b). Up to 18% of consecutive endoscopy patients were found to harbour this aberration (Spechler et al. 1994a), but it is uncertain how much the macroscopic misclassification discussed in our present paper may have contributed. Although claimed to be a manifestation of gastro-oesophageal reflux disease (Oberg et al. 1997b), this type of metaplasia remains to be defined in terms of subsequent prognosis, including adenocarcinoma risk. Therefore, it is typically not classified as Barrett's oesophagus. Accordingly, some of our "false negative" cases may not have been false negative vis-à-vis genuine Barrett's oesophagus.

10.2.4 Risk factors for Barrett's oesophagus

In this study, designed to come close to a truly population-based epidemiological evaluation, reflux symptoms, age, and smoking emerged as risk factors for Barrett's oesophagus. Body mass index appeared to be unrelated to risk. Unexpectedly, we noted a complex relationship between reflux, subjunctional presence of *H. pylori,* and BO.

Most of our results are supported by several of the published hospital-based studies which made comparisons with mixed endoscopy room or hospital controls. The association of BO with reflux symptoms or other clinical markers of GORD and/or GORD severity has been a consistent finding in most (Eisen et al. 1997; Voutilainen et al. 2000; Conio et al. 2002; Lieberman et al. 2004), but not all (Hirota et al. 1999). It is notable, however, that approximately half of our case patients did not fulfil our criteria for frequent reflux. Although the reflux symptoms tend to subside as columnar metaplasia develops (Ter and Castell 1997), the combined data suggest that reflux disease may not be a necessary cause.

In line with our finding, age was positively linked to the probability of having BO in a majority of the relevant previous studies (Cameron 1999; Hirota et al. 1999; Voutilainen et al. 2000; Lieberman et al. 2004), albeit some other showed no (Vicari et al. 1998) or even a weak inverse association (Conio et al. 2002). The positive relationship with age is consistent with a

continuous replenishment of new cases to the prevalence pool, despite the rarity with which development of BO is actually observed during endoscopic surveillance.

Our statistically non-significant risk elevation for BO linked to female sex is at odds with virtually all previous literature (Vicari et al. 1998; Cameron 1999; Hirota et al. 1999; Voutilainen et al. 2000; Conio et al. 2002; Lieberman et al. 2004), although the male predominance was marginal in one of the studies of endoscopy patients (Vicari et al. 1998) and in the population-based Swedish study (Ronkainen et al. 2005). Notwithstanding that our study might have some epidemiological advantages, the absence of statistical significance and lack of consistence hint that our observed female predominance is a chance finding. However, the combined literature suggests that the male predominance is less in BO than in oesophageal adenocarcinoma (Lagergren et al. 1999a), indicating that the probability of progression from already established BO to cancer is higher in men than in women.

We found no clear relationship of BO with body mass, one of the strongest risk factors for oesophageal adenocarcinoma. This seemingly paradoxical result is in good agreement with the findings of others (Ritenbaugh et al. 1995; Cameron 1999), including the recent population-based study by Ronkainen et al (Ronkainen et al. 2005), although one previous retrospective hospital-based study showed an association between overweight and BO (Stein et al. 2005). The causal role of obesity in GORD has been debated (Lagergren et al. 2000c; Nandurkar et al. 2004); in a previous population-based study in Sweden (Lagergren et al. 2000c), we failed to demonstrate a positive relationship between BMI and occurrence of reflux symptoms. This finding has been contradicted by other studies (Nandurkar et al. 2004), but overall it appears that the relationship between body mass and GORD is unimpressive. Hence, the presumed oesophageal carcinogenicity associated with body mass seems to be exerted predominantly after BO development.

Our observed positive association with tobacco smoking gets support from two studies (Eisen et al. 1997; Hirota et al. 1999), while the association disappeared after multivariable adjustments in one (Conio et al. 2002). In a fourth, small study (Ritenbaugh et al. 1995), cigarette smoking was weakly positively associated with the risk of having long-segment BO, but weakly negatively associated with the presence of the short-segment variant, while the use of other tobacco products was positively linked to both variants. Smoking has been consistently, although not very strongly, associated with risk of oesophageal adenocarcinoma in most studies (Lagergren et al. 2000b; Enzinger and Mayer 2003). In the causal pathway, it appears that smoking acts mainly at steps preceding BO development.

Most investigators addressing alcohol as a risk factor in BO patients compared with non-GORD controls found weak and non-significant relationships (Hirota et al. 1999; Conio et al. 2002). A study among US Veterans revealed a considerably higher consumption of hard liquor and mixed drinks among the BO cases (Ritenbaugh et al. 1995), in conflict with our observed negative – but statistically non-significant – relationship. As alcohol consumption is unrelated to risk for oesophageal adenocarcinoma (Lagergren et al. 2000b), an important causal role in BO appears implausible.

Our most controversial finding is the seemingly complex relationship between presence of *H. pylori* in the cardia, reflux, and BO. Overall, subjunctional presence of *H. pylori* showed no clear relation with oesophageal metaplasia in our study; it tended to be weakly inversely associated with MCM but weakly positively associated with BO. In the absence of reflux, however, it was inversely linked to both MCM and BO, albeit statistically non-significant.

This is in agreement with the findings of the only previous study that specifically correlated subjunctional presence of *H. pylori* with occurrence of BO (Hirota et al. 1999), but it is also in line with the studies that measured presence of *H. pylori* anywhere in the stomach, either histologically (Voutilainen et al. 2000) or serologically (Vicari et al. 1998). One recent study, however, reported significantly higher serologic *H. pylori* prevalence among BO patients compared to controls (Ferrandez et al. 2006). The inverse association between *H. Pylori* and BO is also consistent with an inverse relationship of *H. pylori* with GORD(Raghunath et al. 2003) and oesophageal adenocarcinoma (Chow et al. 1998a; Henrik Siman et al. 2001; Ye et al. 2004; de Martel et al. 2005). Our observation of a strong interaction between subjunctional presence of *H. pylori* and reflux, so that these two factors were intimately linked with BO only when they occurred in combination, has not been reported previously. This finding is hard to reconcile with an inverse overall relationship between *H. pylori* and oesophageal adenocarcinoma. A conceivable explanation would be that a sizeable proportion of the cases classified as having short segment BO, in fact, did not have this condition, and that the biopsies above the OGJ were mistakenly taken below this landmark. Since IM below the GOJ was positively linked to presence of *H. pylori*, both in our study and in others' (Hirota et al. 1999; Voutilainen et al. 2000), such misclassification would lead to a spurious positive association between subjunctional *H. pylori* and BO. The interaction with reflux could arise if this misclassification occurred predominantly in individuals with reflux symptoms, for instance in those with hiatal hernias. On the other hand, all examinations in the present study were performed by experienced endoscopists and in accordance with a protocol where the anatomical landmarks were emphasized. Further, if misclassification of short segment BO would be the explanation, one would expect the prevalence of this condition to be higher than in other studies, but it was not (Spechler et al. 1994a; Hirota et al. 1999; Johansson et al. 2005). Moreover, we found no strong positive overall association between subjunctional *H. pylori* and BO. This would be expected if the proposed misclassification would have been a prominent feature of our study; also, the positive association remained (however still not statistically significant) in analyses where cases were restricted to BO $\geq$ 2 cm. It should be emphasized that the interaction was with colonization of the microorganism in the subjunctional cardia and not necessarily with its presence anywhere in the stomach. Our interpretation must, however, be cautious; before speculating about mechanisms and possible clinical implications, our finding needs to be confirmed by others. A complex relationship between *H. pylori* and BO is not totally inconceivable; in a recent Italian study, presence of duodenal ulcer – in turn closely linked with *H. pylori* infection – was positively associated with the occurrence of BO (Conio et al. 2002).

The risk factor profile for Barrett's oesophagus based on the present study in relation to previous and current literature is summarized in Table 16.

Table 16. Summary of the risk factor profile of Barrett's oesophagus with regard to the results of the present study and related to previous and current literature.

Risk factor	Barrett's oesophagus
Age	+
Male sex	+ (?)
Gastro-oesophageal reflux	++
Body mass	- (?)
Tobacco smoking	+
Alcohol use	-
Helicobacter pylori	-/+ (?)

+ denotes a weak positive association, ++ denotes a moderately strong positive association and – denotes absence of a significant association.

10.2.5 Pancreatic acinar metaplasia

Our study confirms that PAM is a common finding among upper gastrointestinal endoscopy patients. PAM did not appear to be randomly distributed across the junctional area; instead the majority of affected individuals had their PAM below the OGJ, while others – particularly women, individuals with subjunctional presence of *H. pylori*, and tentatively those with indications of GORD – had their PAM located above this anatomical landmark. More than one third of an admittedly small number of patients with Barrett's oesophagus exhibited such PAM. No more than a minority had PAM on both sides of the OGJ.

Since we took biopsies immediately above and below the OGJ but not exactly at the squamocolumnar junction we might have missed some cases, explaining our somewhat lower overall prevalence compared to a previous American study (Wang et al. 1996). Our observed prevalence of PAM below the OGJ is more in line with 9% reported by Doglioni et al (Doglioni et al. 1993), but lower than that reported in a small American study in patients with GORD and a mixed control group of healthy volunteers and patients with a variety of gastroduodenal diseases (el-Zimaity et al. 2000). The high prevalence of PAM above the OGJ (38%) observed among our patients with Barrett's oesophagus was not significantly different from the 18% reported by Wang et al (Wang et al. 1996), but considerably higher than the 7% noted in an American drug treatment trial which also included Barrett's mucosa of fundic and junctional type (Krishnamurthy and Dayal 1995). In our study oesophageal columnar metaplasia by endoscopy alone (irrespective of histological mucosa type), and by histology alone (irrespective of endoscopic finding) also harboured PAM in 28% and 23% of the

patients, respectively. Thus, PAM is not only occurring in the gastric cardia but is also a common finding in the columnar lined distal oesophagus, and according to our data seemingly most prevalent in patients with an endoscopically and histologically confirmed (intestinal metaplasia) diagnosis of Barrett's oesophagus.

Our borderline association between age and PAM above and/or below the OGJ was not supported by other studies (Wang et al. 1996; el-Zimaity et al. 2000). In fact, Wang et al reported patients with PAM at the OGJ to be significantly younger than patients without PAM, and in another retrospective series investigating PAM in the gastric cardia no correlations with age were found (el-Zimaity et al. 2000). However neither of these studies had the same clear anchorage in the underlying general population as had our study. Interestingly, a study on paediatric upper gastrointestinal endoscopy patients (median age 13 yrs, range 1 day-18 yrs, GORD related symptoms present in 46%) found pancreatic acinar epithelium in the gastric cardia in 16% of the patients (Glickman et al. 2002). Taken together with data from a study that reported a 100% prevalence of PAM when gastrectomy specimens were sampled exhaustively (Doglioni et al. 1993), there has been some support for the view that this lesion might be congenital. However in the paediatric study GORD related symptoms were present in 46% of the cases (Glickman et al. 2002), and the indication for gastrectomy in the second study was gastric cancer, which is strongly correlated to *H. pylori* (Malfertheiner et al. 2005). Thus, previous data do not exclude the possibility that PAM might be an effect of certain exposures. Even if PAM would be congenital and detectable when the sampling density is sufficiently high, our finding is consistent with an age-dependent expansion of PAM in the OGJ area. A similar expansion seems to occur also for cardia type mucosa in this region (Johansson et al. 2005).

We found female sex to be inversely associated (borderline significant) with PAM below the OGJ, while a positive association was at hand with regard to PAM above the OGJ. This finding is at odds with that of an American endoscopy-room-based study (Wang et al. 1996). In the absence of a biologically plausible explanation for the sex difference, we cannot exclude the possibility that this difference might be a chance finding.

Whilst none of the studied covariates – apart from age – seemed to explain the variation in occurrence of PAM below the OGJ, independent associations with PAM above the OGJ, in addition to age and sex, emerged for both reflux symptoms and subjunctional presence of *Helicobacter pylori*. Out of three previous studies investigating the correlation between *H. pylori* and PAM in the OGJ area one found a strong association between PAM in the cardia and *H. pylori* (el-Zimaity et al. 2000), while the two others found no such association (Wang et al. 1996; Polkowski et al. 2000). All but one (Wang et al. 1996) of these studies, however, investigated selected groups of patients, e.g. patients undergoing oesophagogastrectomy (Polkowski et al. 2000). PAM was also reported to be unrelated to "upper gastrointestinal tract disease" (Wang et al. 1996) and "gastro-oesophageal disease" (el-Zimaity et al. 2000).

Our differential results with regard to predictors of PAM above and below the OGJ is in accordance with a concept of different aetiologies of mucosal disease, notably intestinal metaplasia, above and below the OGJ (Spechler 2004).

In our study PAM was exclusively seen in biopsies with cardia type mucosa or intestinal metaplasia, which is in accordance with the study by Doglioni et al (Doglioni et al. 1993) who found a significant correlation to intestinal and "pyloric" types of metaplasia within the gastric mucosa. This might raise the concern that our observed associations are driven mainly

by presence or absence of such mucosa. This concern is allayed by additional analyses restricted to patients with cardia type and/or intestinal metaplasia above the OGJ, and analyses adjusted for intestinal metaplasia above the OGJ, in which the associations of PAM above the OGJ with female gender, reflux symptoms and *H. pylori* remained essentially unchanged (data not shown).

Oesophageal pH monitoring in patients with PAM has not, to our knowledge, been reported previously. For ethical and economical reasons, only a small sample of volunteering patients underwent such testing, so the precision in our data is very limited. Our results are consistent with the observed associations of PAM above the OGJ with both reflux symptoms and Barrett's oesophagus. However, due to the low numbers, cautious interpretation is warranted.

11 CONCLUSIONS

- Among unselected Swedish gastroscopy patients generated from a defined population Barrett's oesophagus is seen in 4%. Intestinal metaplasia overall in the cardia and distal oesophagus is seen in 14% and in a "normal-looking" oesophagogastric junction in 11%.

- Among unselected consecutive new endoscopy patients in a routine clinical setting, the agreement between the macroscopic and microscopic evaluations in the diagnosis of Barrett's oesophagus is no more than fair, and partly dependent on the presence of patient characteristics suggestive of pathology in this region.

- The dominating – but not obligatory – role of reflux in the aetiology of BO is confirmed. High age and tobacco smoking are additional risk factors, but body mass appears to be unrelated to risk. An observed strong amplification of the effects of reflux on BO risk by subjunctional presence of *H. pylori* calls for confirmation by other investigators.

- Among Swedish gastroscopy patients pancreatic acinar metaplasia is found in every 5th patient and associations of PAM above the OGJ with age, subjunctional presence of *H. pylori*, and manifestations of GORD (reflux symptoms, 24h pH pattern and Barrett's oesophagus) suggest that the lesions are acquired and/or expanded with increasing exposure time. Its clinical significance, however, remain obscure.

12 SOME FUTURE PERSPECTIVES

The underlying cause(s) for the oesophageal adenocarcinoma incidence increase are not unveiled, despite huge efforts.The role of Barrett's oesophagus in this scenario is not clear and valid population-based data on Barrett epidemiology are still scarce and low in numbers. Are the strong driving forces to be sought before or after Barrett development? Or by the side of the columnar metaplasia? There are still many questions with regard to the cancer risk in patients with oesophageal intestinal metaplasia, and apart from dysplasia there are no valid and useful risk markers. How should we avoid large scale surveillance in low, or even no risk, patients?

The role of *Helicobacter pylori* (and maybe of other potential microorganisms) in Barrett's oesophagus needs further investigation. What interacting factors drive the reflux-associated metaplasia development?

Pancreatic acinar metaplasia in the oesophagogastric junction area deserves further attention. Is there really no clinical significance in this very distinctive histological feature that seems to be related to conditions with malignant potential?

13 SVENSK SAMMANFATTNING (SWEDISH SUMMARY)

De senaste årtiondena har matstrupscancer av körteltyp ökat dramatiskt i västvärlden. Vad detta beror på vet man inte, men man vet att personer som är kraftigt överviktiga och personer som besväras av sura uppstötningar och halsbränna löper en ökad risk att utveckla denna cancer. Barretts matstrupe är ett förstadium till denna cancerform och upptäcks, ofta av en händelse, i samband med kikarundersökning av magsäcken (gastroskopi), vilket utförs på personer där man p g a skiftande besvär misstänker sjukdomar i matstrupen, magsäcken eller tolvfingertarmen. Mot bakgrund ökningen av körtelcancer i matstrupen ville vi undersöka hur vanligt detta förstadium är, vad det beror på och vilka faktorer som har betydelse vid själva fastställandet av diagnosen (vilket kräver både gastroskopi, samt vävnadsprov i samband med denna).

Vi undersökte 769 patienter som kom för sin första gastroskopiundersökning. Vävnadsprover togs och patienterna fick också besvara ett frågeformulär om längd, vikt, halsbränna, sura uppstötningar, rökning och alkohol. Även ett slumpmässigt urval kontrollpersoner ur den vanliga befolkningen fick besvara detta frågeformulär. Vi hittade Barretts matstrupe hos var 25:e patient och dessa patienter var äldre, var oftare kvinnor, hade mer besvär av halsbränna och sura uppstötningar samt var i högre grad rökare än jämförbara patienter utan denna förändring i matstrupen. Eftersom halsbränna och sura uppstötningar även ökar risken för körtelcancer i matstrupen var detta inte förvånande. Att de flesta patienter med Barretts matstrupe visade sig vara kvinnor var däremot oväntat eftersom de som insjuknar i denna cancerform till allra största delen är män. Ett annat oväntat fynd var att magsårsbakterier (*Helicobacter pylori*) tycktes öka risken för Barretts matstrupe ytterligare hos de patienter som angett besvär av halsbränna och sura uppstötningar. Detta är egendomligt eftersom det finns mycket som talar för att dessa bakterier skyddar mot denna typ av matstrupscancer. Dessa båda fynd måste dock tolkas med försiktighet och behöver bekräftas ytterligare innan några säkra slutsatser kan dras. I förhållande till alkohol och övervikt såg vi inga övertygande samband med Barretts matstrupe.

När det gäller diagnostiken av Barretts matstrupe gav vår studie hållpunkter för att om en patient har besvär eller uppvisar sjukdomstillstånd som gör att man har anledning att misstänka Barretts matstrupe så är undersökaren (som gör gastroskopin) mycket mer alert, och chansen är större att upptäcka denna förändring, som ibland kan vara diskret och svår att skilja från ett mer normalt utseende. Överlag var dock överensstämmelsen mellan den bild man uppfattade i kikarinstrumentet och det man sedan såg i mikroskopet vid undersökningen av vävnadsprovet inte särskilt god, om man tog hänsyn till slumpens inverkan. Detta skulle dock delvis kunna förklaras av att det är svårt att ta bra vävnadsprover från övergången mellan matstrupe och magsäck.

I samband med denna studie noterade vi också en annan mikroskopisk slemhinneförändring, som man inte vetat vad den har för betydelse. Detta är en förändring som har likheter med bukspottkörtelvävnad och som också visat sig producera spjälkningsämnen (enzymer) på plats i matstrupen. När vi undersökte denna förändring närmare fann vi att ca var 5:e patient hade denna i matstrupen eller i övre delen av magsäcken. När den var belägen i matstrupen var den ofta förknippad med magsårsbakterier och fanns i högre grad hos kvinnor än hos män. Det visade sig också vid vår undersökning att dessa patienter i genomsnitt hade mätbar uppstötning av magsyra i matstrupen längre tid per dygn än vad en normal person brukar ha. Vilken betydelse dessa fynd har för eventuell sjukdomsutveckling kan vi inte säga, och huruvida den tillhör normaltillståndet är ännu inte klarlagt.

14 ACKNOWLEDGEMENTS

First I want to thank my wife **Lena** for your faithful love and your patience with my research efforts. I also want to thank my lovely daughter **Julia**, for making me warm and happy inside, and together with my wife giving me perspective on my life in science and medicine.
Further I want to thank my **mother** and **father** for love and great support in many different aspects.

I also want to express my sincere gratitude to:

Olof Nyrén, my main supervisor, for your belief in my ability, for sharing your true scientific interest and excellence, and for an always open door to your office.

Hans-Olof Håkansson, my co-supervisor during the Ph D studies, and supervisor during my training in surgery, for continuous enthusiasm and encouragement to move forward.

Lennart Mellblom, my co-supervisor, for serious support in many aspects.

Hans-Olov Adami, for generous atmosphere and inspiring leadership at MEB.

Fredrik Granath, my co-author, for essential help in different biostatistical matters.

Karl-Erik Johansson, my co-author, for many very nice contacts personal, by letter and by phone, always willing to share your experience as a clinician and researcher.

Antti Kempas, my co-author, for your contributions to my thesis work and your most friendly support.

Lars Brudin and **Gerhard Kjellén**, co-authors of my fourth manuscript, for help and support.

Lars Lundell and **Johan Permert**, professors at ÖAK, Karolinska University Hospital, Huddinge for the opportunity to experience an academic surgical unit, and for valuable discussions about life and work.

Bengt Hjelmqvist and **Carl-Erik Nordgren** former and current heads of the Department of Surgery in Kalmar, for interest and financial support, and all colleagues at the Department of Surgery, Kalmar, that performed the endoscopies of the study.

Gun Olsén and **Susanne Kjellin** for coordinating the endoscopies in Kalmar and Växjö.

Agneta Hansson for handling of data and **Joakim Vang** for database design

Solvig Linder, for helpful coordination of results from the 24 pH metry studies in Kalmar.

Leila Nyrén, for help with data input.

Bahram Rashidkhani for many interesting talks about almost everything.

Kazem Zendehdel and **Shahram Bahmanyar**, my fellow doctoral students in the post-room corridor, for many stimulating discussions about life, science and God.

Johan Askling, former Ph D student at MEB, for kindly helping me with my first steps in SAS programming.

Weimin Ye, for your willingness to give a helping hand, especially in SAS programming.

Catarina Jansson, former Ph D student at MEB, for many fruitful and interesting discussions in science and other aspects.

Karin Ekström Smedby, **Lena Rosenberg**, **Mårten Kivi**, **Maria Held**, **Micaela Prochazka Michael Fored**, **Magnus Kaijser**, **Olof Stephansson**, **Lena George**, **Mikael Hartman** and many others at the department for nice friendship.

Last but not the least, my dear brother **Peter** and my aunt **Barbro** for continuing and encouraging interest in the progress of my research projects, manuscripts and articles.

15 REFERENCES

Ackermark P, Kuipers EJ, Wolf C, Breumelhof R, Seldenrijk CA, Timmer R, Segeren KC, Kusters JG and Smout AJ. Colonization with cagA-positive Helicobacter pylori strains in intestinal metaplasia of the esophagus and the esophagogastric junction. Am J Gastroenterol 2003;98:1719-24.

Adler RH. The lower esophagus lined by columnar epithelium. Its association with hiatal hernia, ulcer, stricture, and tumor. J Thorac Cardiovasc Surg 1963;45:13-34.

Allison PR and Johnstone AS. The oesohagus lined with gastric mucous membrane. Thorax 1953;8:87-101.

Altman D. Practical statistics for medical research. London, Chapman and Hall; 1991.

Banki F, Demeester SR, Mason RJ, Campos G, Hagen JA, Peters JH, Bremner CG and Demeester TR. Barrett's Esophagus in Females: A Comparative Analysis of Risk Factors in Females and Males. Am J Gastroenterol 2005;100:560-7.

Barrett NR. Chronic peptic ulcer of the oesophagus and "oesophagitis". Br J Surg 1950;38:175-82.

Barrett NR. The lower oesophagus lined by columnar epithelium. Surgery 1957;41:881-94.

Bland M. An Introduction to Medical Statistics. Oxford, Oxford University Press; 2000.

Blot WJ, Devesa SS, Kneller RW and Fraumeni JF, Jr. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. Jama 1991;265:1287-9.

Botterweck AA, Schouten LJ, Volovics A, Dorant E and van Den Brandt PA. Trends in incidence of adenocarcinoma of the oesophagus and gastric cardia in ten European countries. Int J Epidemiol 2000;29:645-54.

Boyer J and Robaszkiewicz M. Guidelines of the French Society of Digestive Endoscopy: monitoring of Barrett's esophagus. The Council of the French Society of Digestive Endoscopy. Endoscopy 2000;32:498-9.

Bremner CG, Lynch VP and Ellis FH, Jr. Barrett's esophagus: congenital or acquired? An experimental study of esophageal mucosal regeneration in the dog. Surgery 1970;68:209-16.

BSG. Guidelines for the diagnosis and management of Barrett's columnar-lined oesophagus. A Report of the Working Party of the British Society of Gastroenterology. Eds., A. Watson, R. C. Heading and N. A. Shepherd. London, BRITTISH SOCIETY OF GASTROENTEROLOGY, http://www.bsg.org.uk; 2005.

Bytzer P, Christensen PB, Damkier P, Vinding K and Seersholm N. Adenocarcinoma of the esophagus and Barrett's esophagus: a population- based study. Am J Gastroenterol 1999;94:86-91.

Cameron AJ. Epidemiology of columnar-lined esophagus and adenocarcinoma. Gastroenterol Clin North Am 1997;26:487-94.

Cameron AJ. Barrett's esophagus: prevalence and size of hiatal hernia. Am J Gastroenterol 1999;94:2054-9.

Cameron AJ. Epidemiology of Barrett's esophagus and adenocarcinoma. Dis Esophagus 2002;15:106-8.

Cameron AJ and Lomboy CT. Barrett's esophagus: age, prevalence, and extent of columnar epithelium. Gastroenterology 1992;103:1241-5.

Cameron AJ, Ott BJ and Payne WS. The incidence of adenocarcinoma in columnar-lined (Barrett's) esophagus. N Engl J Med 1985;313:857-9.

Cameron AJ, Zinsmeister AR, Ballard DJ and Carney JA. Prevalence of columnar-lined (Barrett's) esophagus. Comparison of population-based clinical and autopsy findings. Gastroenterology 1990;99:918-22.

Campos GM, DeMeester SR, Peters JH, Oberg S, Crookes PF, Hagen JA, Bremner CG, Sillin LF, 3rd, Mason RJ and DeMeester TR. Predictive factors of Barrett esophagus: multivariate analysis of 502 patients with gastroesophageal reflux disease. Arch Surg 2001;136:1267-73.

Caygill CP, Johnston DA, Lopez M, Johnston BJ, Watson A, Reed PI and Hill MJ. Lifestyle factors and Barrett's esophagus. Am J Gastroenterol 2002;97:1328-31.

Chandrasoma PT, Der R, Ma Y, Dalton P and Taira M. Histology of the gastroesophageal junction: an autopsy study. Am J Surg Pathol 2000a;24:402-9.

Chandrasoma PT, Lokuhetty DM, Demeester TR, Bremmer CG, Peters JH, Oberg S and Groshen S. Definition of histopathologic changes in gastroesophageal reflux disease. Am J Surg Pathol 2000b;24:344-51.

Chow WH, Blaser MJ, Blot WJ, Gammon MD, Vaughan TL, Risch HA, Perez-Perez GI, Schoenberg JB, Stanford JL, Rotterdam H, West AB and Fraumeni JF, Jr. An inverse relation between cagA+ strains of Helicobacter pylori infection and risk of esophageal and gastric cardia adenocarcinoma. Cancer Res 1998a;58:588-90.

Chow WH, Blot WJ, Vaughan TL, Risch HA, Gammon MD, Stanford JL, Dubrow R, Schoenberg JB, Mayne ST, Farrow DC, Ahsan H, West AB, Rotterdam H, Niwa S and Fraumeni JF, Jr. Body mass index and risk of adenocarcinomas of the esophagus and gastric cardia. J Natl Cancer Inst 1998b;90:150-5.

Coad RA and Shepherd NA. Barrett's oesophagus: Definition, diagnosis and pathogenesis. Current Diagnostic Pathology 2003;9:218-227.

Coenraad M, Masclee AA, Straathof JW, Ganesh S, Griffioen G and Lamers CB. Is Barrett's esophagus characterized by more pronounced acid reflux than severe esophagitis? Am J Gastroenterol 1998;93:1068-72.

Conio M, Filiberti R, Blanchi S, Ferraris R, Marchi S, Ravelli P, Lapertosa G, Iaquinto G, Sablich R, Gusmaroli R, Aste H and Giacosa A. Risk factors for Barrett's esophagus: A case-control study. Int J Cancer 2002;97:225-229.

Connor MJ and Sharma P. Chromoendoscopy and magnification endoscopy in Barrett's esophagus. Gastrointest Endosc Clin N Am 2003;13:269-77.

Corley DA and Buffler PA. Oesophageal and gastric cardia adenocarcinomas: analysis of regional variation using the Cancer Incidence in Five Continents database. Int J Epidemiol 2001;30:1415-25.

Crew KD and Neugut AI. Epidemiology of upper gastrointestinal malignancies. Semin Oncol 2004;31:450-64.

Csendes A, Smok G, Burdiles P, Korn O, Gradiz M, Rojas J and Recio M. Prevalence of intestinal metaplasia according to the length of the specialized columnar epithelium lining the distal esophagus in patients with gastroesophageal reflux. Dis Esophagus 2003;16:24-8.

de Martel C, Llosa AE, Farr SM, Friedman GD, Vogelman JH, Orentreich N, Corley DA and Parsonnet J. Helicobacter pylori infection and the risk of development of esophageal adenocarcinoma. J Infect Dis 2005;191:761-7.

Devesa SS, Blot WJ and Fraumeni JF, Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. Cancer 1998;83:2049-53.

Dibble C, Levine MS, Rubesin SE, Laufer I and Katzka DA. Detection of reflux esophagitis on double-contrast esophagrams and endoscopy using the histologic findings as the gold standard. Abdom Imaging 2004;29:421-5.

Doglioni C, Laurino L, Dei Tos AP, De Boni M, Franzin G, Braidotti P and Viale G. Pancreatic (acinar) metaplasia of the gastric mucosa. Histology, ultrastructure, immunocytochemistry, and clinicopathologic correlations of 101 cases. Am J Surg Pathol 1993;17:1134-43.

Eisen GM, Lieberman D, Fennerty MB and Sonnenberg A. Screening and surveillance in Barrett's esophagus: a call to action. Clin Gastroenterol Hepatol 2004;2:861-4.

Eisen GM, Sandler RS, Murray S and Gottfried M. The relationship between gastroesophageal reflux disease and its complications with Barrett's esophagus. Am J Gastroenterol 1997;92:27-31.

Ekstrom AM, Hansson LE, Signorello LB, Lindgren A, Bergstrom R and Nyren O. Decreasing incidence of both major histologic subtypes of gastric adenocarcinoma--a population-based study in Sweden. Br J Cancer 2000;83:391-6.

Eloubeidi MA and Provenzale D. Does this patient have Barrett's esophagus? The utility of predicting Barrett's esophagus at the index endoscopy. Am J Gastroenterol 1999;94:937-43.

el-Zimaity HM, Verghese VJ, Ramchatesingh J and Graham DY. The gastric cardia in gastro-oesophageal disease. J Clin Pathol 2000;53:619-25.

Endlicher E, Rummele P, Beer S, Knuchel R, Rath H, Schlottmann K, Grossmann J, Woenckhaus U, Scholmerich J and Messmann H. Barrett's Esophagus: a Discrepancy between Macroscopic and Histological Diagnosis. Endoscopy 2005a;37:1131-5.

Endlicher E, Rummele P, Beer S, Knuchel R, Rath H, Schlottmann K, Grossmann J, Woenckhaus U, Scholmerich J and Messmann H. Barrett's Esophagus: a Discrepancy between Macroscopic and Histological Diagnosis. Endoscopy. 2005b;37:1131-5.

Enzinger PC and Mayer RJ. Esophageal cancer. N Engl J Med 2003;349:2241-52.

Falk GW. Barrett's esophagus. Gastroenterology 2002;122:1569-91.

Farrow DC, Vaughan TL, Hansten PD, Stanford JL, Risch HA, Gammon MD, Chow WH, Dubrow R, Ahsan H, Mayne ST, Schoenberg JB, West AB, Rotterdam H, Fraumeni JF, Jr. and Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998;7:97-102.

Farrow DC, Vaughan TL, Sweeney C, Gammon MD, Chow WH, Risch HA, Stanford JL, Hansten PD, Mayne ST, Schoenberg JB, Rotterdam H, Ahsan H, West AB, Dubrow R, Fraumeni JF, Jr. and Blot WJ. Gastroesophageal reflux disease, use of H2 receptor antagonists, and risk of esophageal and gastric cancer. Cancer Causes Control 2000;11:231-8.

Ferrandez A, Benito R, Arenas J, Garcia-Gonzalez MA, Sopena F, Alcedo J, Ortego J, Sainz R and Lanas A. CagA-positive Helicobacter pylori infection is not associated with decreased risk of Barrett's esophagus in a population with high H. pylori infection rate. BMC Gastroenterol. 2006;6:7.

Flejou JF. Barrett's oesophagus: from metaplasia to dysplasia and cancer. Gut 2005;54:i6-i12.

Gammon MD, Schoenberg JB, Ahsan H, Risch HA, Vaughan TL, Chow WH, Rotterdam H, West AB, Dubrow R, Stanford JL, Mayne ST, Farrow DC, Niwa S, Blot WJ and Fraumeni JF, Jr. Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. J Natl Cancer Inst 1997;89:1277-84.

Gerson LB, Shetler K and Triadafilopoulos G. Prevalence of Barrett's esophagus in asymptomatic individuals. Gastroenterology 2002;123:461-7.

Glickman JN, Fox V, Antonioli DA, Wang HH and Odze RD. Morphology of the Cardia and Significance of Carditis in Pediatric Patients. Am J Surg Pathol 2002;26:1032-1039.

Glickman JN, Wang H, Das KM, Goyal RK, Spechler SJ, Antonioli D and Odze RD. Phenotype of Barrett's esophagus and intestinal metaplasia of the distal esophagus and gastroesophageal junction: an immunohistochemical study of cytokeratins 7 and 20, Das-1 and 45 MI. Am J Surg Pathol 2001;25:87-94.

Goldblum JR, Richter JE, Vaezi M, Falk GW, Rice TW and Peek RM. Helicobacter pylori infection, not gastroesophageal reflux, is the major cause of inflammation and intestinal metaplasia of gastric cardiac mucosa. Am J Gastroenterol 2002;97:302-11.

Goldblum JR, Vicari JJ, Falk GW, Rice TW, Peek RM, Easley K and Richter JE. Inflammation and intestinal metaplasia of the gastric cardia: the role of gastroesophageal reflux and H. pylori infection. Gastroenterology 1998;114:633-9.

GOSPE. Barrett's esophagus: epidemiological and clinical results of a multicentric survey. Gruppo Operativo per lo Studio delle Precancerosi dell'Esofago (GOSPE). Int J Cancer 1991;48:364-8.

Haggitt RC, Tryzelaar J, Ellis FH and Colcher H. Adenocarcinoma complicating columnar epithelium-lined (Barrett's) esophagus. Am J Clin Pathol 1978;70:1-5.

Hakansson HO, Mellblom L, Johansson J, Bjartell A and Borgstrom A. Synthesis and localization of pancreatic secretory proteins in pancreatic acinar-like metaplasia in the distal part of the oesophagus. Pancreatic acinar metaplasia: another source of pancreatic enzymes! Scand J Gastroenterol 2003;38:10-3.

Hansson LE, Bergstrom R, Sparen P and Adami HO. The decline in the incidence of stomach cancer in Sweden 1960-1984: a birth cohort phenomenon. Int J Cancer 1991;47:499-503.

Hansson LE, Sparen P and Nyren O. Increasing incidence of both major histological types of esophageal carcinomas among men in Sweden. Int J Cancer 1993;54:402-7.

Hawe A, Payne WS, Weiland LH and Fontana RS. Adenocarcinima in the columnar epithelial lined lower (Barret) oesophagus. Thorax. 1973;28:511-4.

Hayward J. The lower end of oesophagus. Thorax 1961;16:36-41.

Henrik Siman J, Forsgren A, Berglund G and Floren CH. Helicobacter pylori infection is associated with a decreased risk of developing oesophageal neoplasms. Helicobacter 2001;6:310-6.

Hirota WK, Loughney TM, Lazas DJ, Maydonovitch CL, Rholl V and Wong RK. Specialized intestinal metaplasia, dysplasia, and cancer of the esophagus and esophagogastric junction: prevalence and clinical data. Gastroenterology 1999;116:277-85.

Hosmer DW and Lemeshow S. Applied Logistic Regression. New York, John Wiley & Sons, Inc.; 1989.

Jankowski JA, Provenzale D and Moayyedi P. Esophageal adenocarcinoma arising from Barrett's metaplasia has regional variations in the west. Gastroenterology 2002;122:588-90.

Jhala NC, Montemor M, Jhala D, Lu L, Talley L, Haber MM and Lechago J. Pancreatic acinar cell metaplasia in autoimmune gastritis. Arch Pathol Lab Med 2003;127:854-7.

Johansson J, Hakansson HO, Mellblom L, Kempas A, Johansson KE, Granath F and Nyren O. Prevalence of precancerous and other metaplasia in the distal oesophagus and gastro-oesophageal junction. Scand J Gastroenterol 2005;40:893-902.

Johansson J, Håkansson H, Mellblom L, Kempas A, Johansson KE, Granath F and Nyren O. Risk factors for Barrett's oesophagus: a population-based approach. Scand J Gastroenterol (In Press).

Johnsson F, Joelsson B and Isberg PE. Ambulatory 24 hour intraesophageal pH-monitoring in the diagnosis of gastroesophageal reflux disease. Gut 1987;28:1145-50.

Johnston MH, Hammond AS, Laskin W and Jones DM. The prevalence and clinical characteristics of short segments of specialized intestinal metaplasia in the distal esophagus on routine endoscopy. Am J Gastroenterol 1996;91:1507-11.

Jones TF, Sharma P, Daaboul B, Cherian R, Mayo M, Topalovski M and Weston AP. Yield of intestinal metaplasia in patients with suspected short-segment Barrett's esophagus (SSBE) on repeat endoscopy. Dig Dis Sci 2002;47:2108-11.

Junqueira LC, Carneiro J and Long JA. Basic Histology. Connecticut, Appleton-Century-Crofts; 1986.

Kilgore SP, Ormsby AH, Gramlich TL, Rice TW, Richter JE, Falk GW and Goldblum JR. The gastric cardia: fact or fiction? Am J Gastroenterol 2000;95:921-4.

Kjellen G, Brudin L and Hakansson HO. Is scintigraphy of value in the diagnosis of gastrooesophageal reflux disease? Scand J Gastroenterol 1991;26:425-30.

Krishnamurthy S and Dayal Y. Pancreatic metaplasia in Barrett's esophagus. An immunohistochemical study. Am J Surg Pathol 1995;19:1172-80.

Lagergren J. Adenocarcinoma of oesophagus: what exactly is the size of the problem and who is at risk? Gut 2005;54 Suppl 1:i1-i5.

Lagergren J. Controversies surrounding body mass, reflux, and risk of oesophageal adenocarcinoma. Lancet Oncol. 2006;7:347-9.

Lagergren J, Bergstrom R, Adami HO and Nyren O. Association between medications that relax the lower esophageal sphincter and risk for esophageal adenocarcinoma. Ann Intern Med 2000a;133:165-75.

Lagergren J, Bergstrom R, Lindgren A and Nyren O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. N Engl J Med 1999a;340:825-31.

Lagergren J, Bergstrom R, Lindgren A and Nyren O. The role of tobacco, snuff and alcohol use in the aetiology of cancer of the oesophagus and gastric cardia. Int J Cancer 2000b;85:340-6.

Lagergren J, Bergstrom R and Nyren O. Association between body mass and adenocarcinoma of the esophagus and gastric cardia. Ann Intern Med 1999b;130:883-90.

Lagergren J, Bergstrom R and Nyren O. No relation between body mass and gastro-oesophageal reflux symptoms in a Swedish population based study. Gut 2000c;47:26-9.

Lieberman D, Fennerty MB, Morris CD, Holub J, Eisen G and Sonnenberg A. Endoscopic evaluation of patients with dyspepsia: results from the national endoscopic data repository. Gastroenterology 2004;127:1067-75.

Lieberman DA, Oehlke M and Helfand M. Risk factors for Barrett's esophagus in community-based practice. GORGE consortium. Gastroenterology Outcomes Research Group in Endoscopy. Am J Gastroenterol 1997;92:1293-7.

Lindblad M, Ye W, Lindgren A and Lagergren J. Disparities in the classification of esophageal and cardia adenocarcinomas and their influence on reported incidence rates. Ann Surg 2006;243:479-85.

Locke GR, 3rd, Talley NJ, Fett SL, Zinsmeister AR and Melton LJ, 3rd. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Olmsted County, Minnesota. Gastroenterology 1997;112:1448-56.

Malfertheiner P, Sipponen P, Naumann M, Moayyedi P, Megraud F, Xiao SD, Sugano K and Nyren O. Helicobacter pylori eradication has the potential to prevent gastric cancer: a state-of-the-art critique. Am J Gastroenterol 2005;100:2100-15.

Mayne ST, Risch HA, Dubrow R, Chow WH, Gammon MD, Vaughan TL, Farrow DC, Schoenberg JB, Stanford JL, Ahsan H, West AB, Rotterdam H, Blot WJ and Fraumeni JF, Jr. Nutrient intake and risk of subtypes of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 2001;10:1055-62.

Miller LS. Endoscopy of the esophagus. The Esophagus. D. O. Castell. Boston, Little, Brown & Co; 1992. 89-142.

Morson BC and Belcher JR. Adenocarcinoma of the oesophagus and ectopic gastric mucosa. Br J Cancer 1952;6:127-30.

Murray L, Watson P, Johnston B, Sloan J, Mainie IM and Gavin A. Risk of adenocarcinoma in Barrett's oesophagus: population based study. Bmj 2003;327:534-5.

Naef AP, Savary M and Ozzello L. Columnar-lined lower esophagus: an acquired lesion with malignant predisposition. Report on 140 cases of Barrett's esophagus with 12 adenocarcinomas. J Thorac Cardiovasc Surg. 1975;70:826-35.

Nam JM. Assessment on homogeneity tests for kappa statistics under equal prevalence across studies in reliability. Stat Med 2006;25:1521-31.

Nandurkar S, Locke GR, 3rd, Fett S, Zinsmeister AR, Cameron AJ and Talley NJ. Relationship between body mass index, diet, exercise and gastro-oesophageal reflux symptoms in a community. Aliment Pharmacol Ther 2004;20:497-505.

Nandurkar S, Talley NJ, Martin CJ, Ng TH and Adams S. Short segment Barrett's oesophagus: prevalence, diagnosis and associations. Gut 1997;40:710-5.

Neumann CS and Cooper BT. 24 hour ambulatory oesophageal pH monitoring in uncomplicated Barrett's oesophagus. Gut 1994;35:1352-5.

Oberg S, Johansson J, Wenner J, Johnsson F, Zilling T, von Holstein CS, Nilsson J and Walther B. Endoscopic surveillance of columnar-lined esophagus: frequency of intestinal metaplasia detection and impact of antireflux surgery. Ann Surg 2001;234:619-26.

Oberg S, Peters JH, DeMeester TR, Chandrasoma P, Hagen JA, Ireland AP, Ritter MP, Mason RJ, Crookes P and Bremner CG. Inflammation and specialized intestinal metaplasia of cardiac mucosa is a manifestation of gastroesophageal reflux disease. Ann Surg 1997a;226:522-30; discussion 530-2.

Oberg S, Peters JH, DeMeester TR, Chandrasoma P, Hagen JA, Ireland AP, Ritter MP, Mason RJ, Crookes P and Bremner CG. Inflammation and specialized intestinal metaplasia of cardiac mucosa is a manifestation of gastroesophageal reflux disease. Ann Surg 1997b;226:522-30; discussion 530-2.

Oberg S, Peters JH, DeMeester TR, Lord RV, Johansson J, DeMeester SR and Hagen JA. Determinants of intestinal metaplasia within the columnar-lined esophagus. Arch Surg 2000;135:651-5; discussion 655-6.

Oberg S, Peters JH, Nigro JJ, Theisen J, Hagen JA, DeMeester SR, Bremner CG and DeMeester TR. Helicobacter pylori is not associated with the manifestations of gastroesophageal reflux disease. Arch Surg 1999;134:722-6.

Padda S and Ramirez FC. Accuracy in the diagnosis of short-segment Barrett's esophagus: the role of endoscopic experience. Gastrointest Endosc 2001;54:605-8.

Paull A, Trier JS, Dalton MD, Camp RC, Loeb P and Goyal RK. The histologic spectrum of Barrett's esophagus. N Engl J Med 1976;295:476-80.

Polkowski W, van Lanschot JJ, ten Kate FJ, Rolf TM, Polak M, Tytgat GN, Obertop H and Offerhaus GJ. Intestinal and pancreatic metaplasia at the esophagogastric junction in patients without Barrett's esophagus. Am J Gastroenterol 2000;95:617-25.

Powell J and McConkey CC. The rising trend in oesophageal adenocarcinoma and gastric cardia. Eur J Cancer Prev 1992;1:265-9.

Powell J, McConkey CC, Gillison EW and Spychal RT. Continuing rising trend in oesophageal adenocarcinoma. Int J Cancer 2002;102:422-7.

Raghunath A, Hungin AP, Wooff D and Childs S. Prevalence of Helicobacter pylori in patients with gastro-oesophageal reflux disease: systematic review. Bmj 2003;326:737.

Reid BJ, Blount PL, Rubin CE, Levine DS, Haggitt RC and Rabinovitch PS. Flow-cytometric and histological progression to malignancy in Barrett's esophagus: prospective endoscopic surveillance of a cohort. Gastroenterology 1992;102:1212-9.

Ritenbaugh C, Sampliner R, Aickin M, Garewal H and Meyskens F. Risk factors for Barrett's oesophagus: a life history approach to behavioural assessment in the distant past. Eur J Cancer Prev 1995;4:459-68.

Ronkainen J, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, Vieth M, Stolte M, Talley NJ and Agreus L. Prevalence of Barrett's esophagus in the general population: an endoscopic study. Gastroenterology. 2005;129:1825-31.

Sampliner RE. Updated guidelines for the diagnosis, surveillance, and therapy of Barrett's esophagus. Am J Gastroenterol 2002;97:1888-95.

Shaheen NJ. Advances in Barrett's Esophagus and Esophageal Adenocarcinoma. Gastroenterology 2005;128:1554-66.

Shaheen NJ, Crosby MA, Bozymski EM and Sandler RS. Is there publication bias in the reporting of cancer risk in Barrett's esophagus? Gastroenterology 2000;119:333-8.

Sharma P. Short segment Barrett esophagus and specialized columnar mucosa at the gastroesophageal junction. Mayo Clin Proc 2001;76:331-4.

Sharma P, Armstrong D and Bergman J. The development and validation of an endoscopic grading system for Barrett's esophagus - the Prague C and M criteria. Gastroenterology 2006a;130:A-121. [Abstract #820].

Sharma P, Falk GW, Weston AP, Reker D, Johnston M and Sampliner RE. Dysplasia and Cancer in a Large Multicenter Cohort of Patients With Barrett's Esophagus. Clin Gastroenterol Hepatol. 2006b.

Sharma P and Sidorenko EI. Are screening and surveillance for Barrett's oesophagus really worthwhile? Gut 2005;54 Suppl 1:i27-i32.

Skinner DB, Walther BC, Riddell RH, Schmidt H, Iascone C and DeMeester TR. Barrett's esophagus. Comparison of benign and malignant cases. Ann Surg 1983;198:554-65.

Solaymani-Dodaran M, Logan RF, West J, Card T and Coupland C. Risk of oesophageal cancer in Barrett's oesophagus and gastro-oesophageal reflux. Gut 2004;53:1070-4.

Spechler SJ. Clinical practice. Barrett's Esophagus. N Engl J Med 2002;346:836-42.

Spechler SJ. Intestinal metaplasia at the gastroesophageal junction. Gastroenterology 2004;126:567-75.

Spechler SJ and Goyal RK. Barrett's esophagus. N Engl J Med 1986;315:362-71.

Spechler SJ and Goyal RK. The columnar-lined esophagus, intestinal metaplasia, and Norman Barrett. Gastroenterology 1996;110:614-21.

Spechler SJ, Zeroogian JM, Antonioli DA, Wang HH and Goyal RK. Prevalence of metaplasia at the gastro-oesophageal junction. Lancet 1994a;344:1533-6.

Spechler SJ, Zeroogian JM, Antonioli DA, Wang HH and Goyal RK. Prevalence of metaplasia at the gastro-oesophageal junction. Lancet 1994b;344:1533-6.

SSAT-AGA-ASGE. Management of Barrett's esophagus. The Society for Surgery of the Alimentary Tract (SSAT), American Gastroenterological Association (AGA), American Society for Gastrointestinal Endoscopy (ASGE) Consensus Panel. J Gastrointest Surg 2000;4:115-6.

Stein DJ, El-Serag HB, Kuczynski J, Kramer JR and Sampliner RE. The association of body mass index with Barrett's oesophagus. Aliment Pharmacol Ther. 2005;22:1005-10.

Sundelof M, Ye W, Dickman PW and Lagergren J. Improved survival in both histologic types of oesophageal cancer in Sweden. Int J Cancer 2002;99:751-4.

Ter RB and Castell DO. Gastroesophageal reflux disease in patients with columnar-lined esophagus. Gastroenterol Clin North Am 1997;26:549-63.

Terry P, Lagergren J, Hansen H, Wolk A and Nyren O. Fruit and vegetable consumption in the prevention of oesophageal and cardia cancers. Eur J Cancer Prev 2001a;10:365-9.

Terry P, Lagergren J, Ye W, Wolk A and Nyren O. Inverse association between intake of cereal fiber and risk of gastric cardia cancer. Gastroenterology 2001b;120:387-91.

Wallner B, Sylvan A, Stenling R and Janunger KG. The esophageal Z-line appearance correlates to the prevalence of intestinal metaplasia. Scand J Gastroenterol 2000;35:17-22.

Walther B and Willen R. [Esophageal cancer on the increase. Is Barrett esophagus the cause?]. Lakartidningen 1996;93:1643-7.

Wang HH, Zeroogian JM, Spechler SJ, Goyal RK and Antonioli DA. Prevalence and significance of pancreatic acinar metaplasia at the gastroesophageal junction. Am J Surg Pathol 1996;20:1507-10.

Vicari JJ, Peek RM, Falk GW, Goldblum JR, Easley KA, Schnell J, Perez-Perez GI, Halter SA, Rice TW, Blaser MJ and Richter JE. The seroprevalence of cagA-positive Helicobacter pylori strains in the spectrum of gastroesophageal reflux disease. Gastroenterology 1998;115:50-7.

Wiener GJ, Richter JE, Copper JB, Wu WC and Castell DO. The symptom index: a clinically important parameter of ambulatory 24-hour esophageal pH monitoring. Am J Gastroenterol 1988;83:358-61.

Winters C, Jr., Spurling TJ, Chobanian SJ, Curtis DJ, Esposito RL, Hacker JF, 3rd, Johnson DA, Cruess DF, Cotelingam JD, Gurney MS and et al. Barrett's esophagus. A prevalent, occult complication of gastroesophageal reflux disease. Gastroenterology 1987;92:118-24.

Wolf C, Seldenrijk CA, Timmer R, Breumelhof R and Smout AJ. Epidemiological, clinical, and histological characteristics of intestinal metaplasia at the oesophagogastric junction compared to short segments of columnar-lined oesophagus. Eur J Gastroenterol Hepatol 2001;13:113-20.

Woolf GM, Riddell RH, Irvine EJ and Hunt RH. A study to examine agreement between endoscopy and histology for the diagnosis of columnar lined (Barrett's) esophagus. Gastrointest Endosc 1989;35:541-4.

Voutilainen M, Farkkila M, Juhola M, Mecklin JP and Sipponen P. Complete and incomplete intestinal metaplasia at the oesophagogastric junction: prevalences and associations with endoscopic erosive oesophagitis and gastritis. Gut 1999a;45:644-8.

Voutilainen M, Farkkila M, Juhola M, Mecklin JP and Sipponen P. Complete and incomplete intestinal metaplasia at the oesophagogastric junction: prevalences and associations with endoscopic erosive oesophagitis and gastritis. Gut 1999b;45:644-8.

Voutilainen M, Farkkila M, Mecklin JP, Juhola M and Sipponen P. Classical Barrett esophagus contrasted with Barrett-type epithelium at normal-appearing esophagogastric junction. Central Finland Endoscopy Study Group. Scand J Gastroenterol 2000;35:2-9.

Voutilainen ME and Juhola MT. The changing epidemiology of esophageal cancer in Finland and the impact of the surveillance of Barrett's esophagus in detecting esophageal adenocarcinoma. Dis Esophagus 2005;18:221-5.

Ye W, Held M, Lagergren J, Engstrand L, Blot WJ, McLaughlin JK and Nyren O. Helicobacter pylori infection and gastric atrophy: risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. J Natl Cancer Inst 2004;96:388-96.

Öberg S, Johansson J, Wenner J, Johnsson F, Zilling T, von Holstein CS, Nilsson J and Walther B. Endoscopic surveillance of columnar-lined esophagus: frequency of intestinal metaplasia detection and impact of antireflux surgery. Ann Surg 2001;234:619-26.