J Gastrointest Surg (2007) 11:479–486
DOI 10.1007/s11605-006-0017-7

Duodeno-Gastric-Esophageal Reflux—What is Pathologic? Comparison of Patients with Barrett's Esophagus and Age-Matched Volunteers

Eva Wolfgarten · Benito Pütz · Arnulf H. Hölscher · Elfriede Bollschweiler

Published online: 19 January 2007

Abstract
Introduction The aim of the study was to analyse pH- and bile-monitoring data in patients with Barrett's esophagus and in age- and gender-matched controls.
Subjects and Methods Twenty-four consecutive Barrett's patients (8 females, 16 males, mean age 57 years), 21 patients with esophagitis (10 females, 11 males, mean age 58 years), and 19 healthy controls (8 females, 11 males, mean age 51 years), were included. Only patients underwent endoscopy with biopsy. All groups were investigated with manometry, gastric and esophageal 24-h pH, and simultaneous bile monitoring according to a standardized protocol. A bilirubin absorption >0.25 was determined as noxious bile reflux. The receiver operator characteristic (ROC) method was applied to determine the optimal cutoff value of pathologic bilirubin levels.
Results Of Barrett's patients, 79% had pathologic acidic gastric reflux (pH<4 >5% of total measuring time). However, 32% of healthy controls also had acid reflux ($p<0.05$) without any symptoms. The median of esophageal bile reflux was 7.8% (lower quartile (LQ)–upper quartile (UQ)=1.6–17.8%) in Barrett's patients, in patients with esophagitis, 3.5% (LQ–UQ= 0.1–13.5), and in contrast to 0% (LQ–UQ=0–1.0%) in controls, $p=0.001$. ROC analysis showed the optimal dividing value for patients at more than 1% bile reflux over 24 h (75% sensitivity, 84% specificity).
Conclusion An optimal threshold to differentiate between normal and pathological bile reflux into the esophagus is 1% (24-h bile monitoring with an absorbance >0.25).

Keywords Reference value bilitec · Bile reflux · Acid reflux · Barrett's mucosa · Esophagus · Spectrophotometry

E. Wolfgarten · B. Pütz · A. H. Hölscher
Department of Visceral and Vascular Surgery,
University of Cologne,
Cologne, Germany

E. Bollschweiler (✉)
Klinik und Poliklinik für Visceral-und Gefäßchirurgie,
der Universität zu Köln,
Kerpener Str. 62,
50937 Köln, Germany
e-mail: Elfriede.bollschweiler@uk-koeln.de

Introduction

The incidence rates for adenocarcinoma (AC) of the esophagus and gastric cardia have risen rapidly in Western industrialized countries.[1] Besides nicotine and alcohol abuse, nutritional factors, high body mass index, acidic gastric reflux, and Barrett's esophagus are believed to be critical factors of carcinogenesis.[2–4] Recent studies have shown that the presence of biliary reflux in combination with acidic gastric reflux damages the esophageal mucosa and causes complications of gastro-esophageal reflux disease (GERD), e.g., development of Barrett's mucosa (BM).[5,6] Duodeno-gastric reflux into the esophagus (DGER), in particular, appears to be important to the pathogenesis of Barrett's esophagus.[7] Prolonged esophageal aspiration studies have documented increased bile acids in patients with severe esophagitis and Barrett's esophagus.[8] Eighty percent of patients with Barrett's

esophagus on proton-pump inhibitors show a normal esophageal pH profile, but 60% show abnormal esophageal exposure to bile as measured by Bilitec 2000.[9]

In the past, direct and prolonged quantification of duodenoesophageal reflux has been difficult to achieve. Now, bilirubin concentration can be directly measured by spectrophotometry, based on the specific absorption at a wavelength of 453 nm. Biliary reflux can be measured with a transnasally passed, ambulatory fiberoptic probe (Bilitec 2000), which records bile absorption. A number of papers have already been published on the exposure of the esophagus[10–13] and stomach[14–16] using this technique. However, in these studies, the control patients were between 25 and 35 years old. In clinical practice, patients with Barrett's mucosa tend to be older. In addition, the authors of each study used varying reference values to measure biliary reflux in the esophagus, making comparison of the measured values difficult.

The aim of the present study was to analyze data of pH and bile monitoring in a collective of healthy age- and gender-matched controls and patients with Barrett's esophagus.

Subjects and Methods

Subjects

Selection of controls was carried out according to a strict protocol. Healthy volunteers treated from 1999 to 2000 between the ages of 40 and 60 years were included in the study. None of the controls were on acid suppressing or gut motility medications, had a history of upper gastrointestinal disease, had undergone upper or major abdominal surgery, or had had therapeutic endoscopic procedures of the upper gastrointestinal tract. Diagnostic endoscopy and barium swallows were not performed, but gallstone disease was excluded by ultrasound scan.

From 1999 to 2002, 24 patients with histologically confirmed Barrett's mucosa were included in the study. For additional comparison, we include a group of patients with esophagitis (stage I to III according Savary and Miller) without Barrett's esophagus, which had the same diagnostic procedures before planned laparoscopic fundoplication. During the aforementioned time span, 21 patients age older than 40 years were available for this study. Exclusion criteria were history of esophageal, gastric, or biliary surgery, history of abdominal or thoracic radiotherapy, or presence of peptic ulcer disease, active gastrointestinal bleeding, esophageal or fundic varices, esophageal or upper small intestine chronic disease, or neoplastic disease. All drugs potentially affecting gastrointestinal motility and secretion were discontinued at least 1 week before the study.

Upper Gastrointestinal Endoscopy

All patients underwent classical upper gastrointestinal endoscopy. If sedation was necessary, intravenous administration of propofol (up to 200 mg) was normally used, or occasionally, midazolam (up to 5 mg) was used. During endoscopy, the presence and extent of esophagitis, Barrett's esophagus, and hiatal hernia was noted. Biopsies were taken from the Barrett's mucosa.

Ambulatory Esophageal/Gastric pH and Bile Monitoring

All groups underwent esophageal manometry and 24-h pH and simultaneous bile monitoring using a standardized protocol. Ambulatory pH monitoring was performed using a transnasally inserted antimony pH electrode with a separate skin reference electrode (Synetics Medical, Stockholm, Sweden). The data were stored on a portable digital recorder (Digitrapper MkIII, Synectics Medical Stockholm, Sweden). Before each study, the pH probe was calibrated in buffer solutions of pH 7 and 1. An episode of acid reflux was defined as a decrease in esophageal pH to less than 4 for more than 10 s.

To quantify duodeno-esophageal reflux, a transnasally passed, ambulatory fiberoptic spectrophotometer (Bilitec 2000, Synectics, Sweden) was used. The system consists of a miniaturized probe of 1.5-mm diameter that carries light signals into the esophagus and back via a plastic fiberoptic bundle. Before each study, the probe was calibrated in water. Corresponding to the current literature, a bilirubin absorption >0.25 was used as a reference for noxious biliary reflux.[14]

The bile and pH probes were taped together and passed transnasally into the esophagus and stomach, as described in detail elsewhere.[17] The upper tips of the probes were positioned 5 cm above the upper border of the lower esophageal sphincter as defined by esophageal manometry. The distal pH electrode and fiberoptic sensor were placed in the fundus of the stomach, 10 cm distal to the lower esophageal sphincter (Fig. 1). Controls and patients were asked to follow a strict protocol of three meals per day, with no liquids between meals. Recumbent phases of recording were permitted only at night. Patients were asked to keep a diary recording of the exact nature of meals, the supine and erect phases of measurement, and the sensations of heartburn and regurgitation.

The simultaneous biliary and pH monitoring was done with administration of a colorless "white diet" (WD) including liquid and solid foods with a maximum in vitro bile absorbance of 0.25 [absorbance scale ranging from 0 (plain water) to 1 (total screen)]. The meals included water, milk, toast, potatoes, chicken, dry biscuits, and fish.

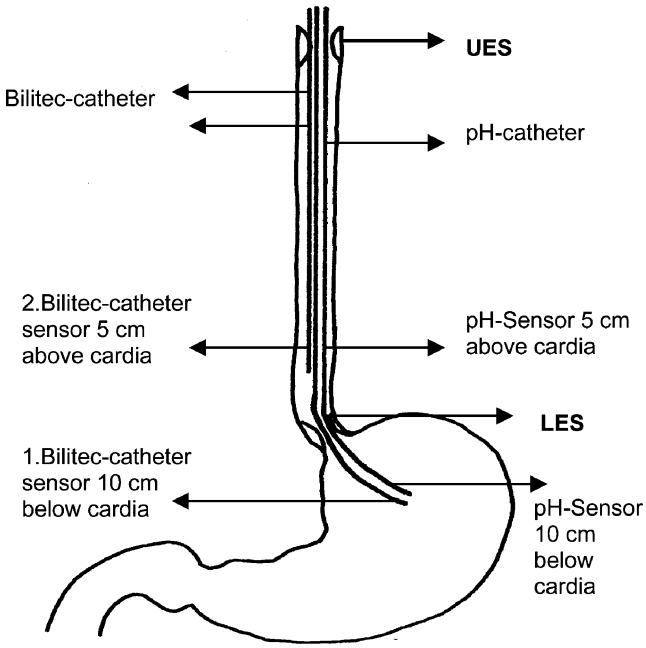

Figure 1 Position of the pH- and bilirubin-probes in the stomach and in the esophagus. (UES=upper esophageal sphincter; LES=lower esophageal sphincter).

Data Acquisition and Interpretation

After completion of the measurements, probes were withdrawn from the patients, and data were stored via interface on an IBM-compatible computer equipped with Polygram® software (Medtronic). The data of each second of the 24-h measurements were used for analysis. To assess the presence of gastric or esophageal biliary reflux, the percentage of time when absorbance was greater than 0.25 was calculated for the following periods: total supine, upright, and postprandial. The postprandial period was defined as 2 h after the end of meals. The percentage of time with esophageal pH lower than 4 and median gastric pH and the percentage of time with gastric pH measuring 1, 2, 3–7, and >7 was also calculated for the above periods. The mean duration of the ambulatory pH and Bilitec monitoring study was 22 h, 40 min in patients and 23 h, 44 min in the controls.

Statistical Analysis

The SPSS (version 11.0, Chicago, Illinois) program was used to analyze the results. For graphical presentation, we used the program MedCalc for Windows, (Version 9.0, MedCalc Software, Belgium). Median, interquartile range (IQR or 25th to 75th percentile) values were established. The nonparametric tests (Mann–Whitney and Kruskal–Wallis analysis) were used to assess the relationship between variables. Box and Whisker plots were used to present some of the data. In these plots, the box represents the IQR, and the Whiskers represent the highest and lowest values. Outliers are also plotted, defined as more than 1.5 times the IQR from the 75th centile. Extreme values were defined as more than three times the IQR from the 75th centile.

A receiver operator characteristic (ROC) curve was used to find a cutoff value for optimal sensitivity and specificity according Zweig and Campbell.[18] The area under the curve (AUC) as a measurement of diagnostic performance of the test was used. The results are given as point with the 95% confidence interval (95% CI) and graphically for presentation of all data. As the positive group, we used the patients with Barrett's mucosa, and the negative test group was defined by the healthy volunteers. A nonparametric distribution of the area under the curve was assumpted.

The assumptions for calculation of the required sample size were alpha=0.05, beta=0.80, and that a test is only valid for daily use if less than 20% of the healthy controls and at least 80% of the patients have positive test results. The calculated sample size for each group was 20.

Ethics

The study protocol was approved by the ethics committee of the University of Cologne. Each subject gave written informed consent.

Results

Twenty-four patients with Barrett's esophagus (mean age: 58 years), 21 patients with esophagitis (mean age: 57 years), and 19 healthy controls with a mean age of 51 years were included in the study. Patients with BM showed esophagitis grade 0 (4 cases), grade I (12 cases), and grade II (8 cases). The control group of patients with esophagitis showed nine cases with grade I, eight cases with grade II, and three cases with grade III. Demographics of patients and volunteers are displayed in Table 1 (the data of one volunteer was not usable due to technical problems).

Acidic Gastric Reflux (AGR)

Patients with Barrett's esophagus, 19 of 24 (79%) and 20 of 21 control patients with esophagitis (95%) had pathologic AGR [pH<4 in >5% of total measuring time (TMT)], but also 6 of the 19 healthy controls (32%) showed pathologic AGR without any symptoms (p=0.002). During the TMT, the median AGR was 10.6% for Barrett patients and 3.2% for controls (p<0.01). In particular, measurements of long acid reflux (LAR), defined as reflux pH<4 lasting longer than 5 min, showed significant differences between patients and controls. Pathologic AGR was found in patients during

Table 1 Demographic Data of Patients with Barrett-Mucosa or Esophagitis and Healthy Volunteers

Parameters	Patients with Barrett's Esophagus (n=24)	Patients with Esophagitis (n=21)	Controls (n=19)	Significance Pat. with Barrett vs Controls
Age (median)	57 years	58 years	51 years	–
Min–max	29–75 years	42–77 years	39–62 years	
Gender m:f	16:8	11:10	11:8	n.s.
BMI (median) min–max	27.0 kg/m^2 (18.6–33.1)	26.9 kg/m^2 (17.9–31.5)	24.1 kg/m^2 (19.62–27.34)	p=0.003
Smokers (%)	n=5 (20.8)	n=4 (19.0)	n=6 (31.6)	n.s.
No alcohol %	n=3 (12.5)	n=5 (23.8)	n=5 (26.3)	n.s.

BMI Body mass index

both the supine and upright fasting measuring periods. In contrast, pathologic AGR in healthy controls occurred only in the upright position (Table 2).

24-h Intragastric pH and Bile Monitoring

Gastric pH monitoring showed no significant differences between patients and controls for all measuring periods (Table 3). Gastric bilirubin exposure, indicating biliary reflux, was significantly more frequent in patients than in controls during all measuring periods (Table 3). Biliary exposure in the supine position typically occurred during the early hours of the morning during sleep, represented by increased absorbance over 2–3 h, with a rapid return to baseline values around the time the subject resumed the upright position. Over the same time period, gastric pH monitoring showed increased pH levels to greater than 2 (Fig. 2).

Bilirubin Exposure of the Esophagus

Over the TMT, the median of esophageal biliary reflux was 7.8% for patients with Barrett's esophagus (LQ–UQ=1.6–17.8%) and 3.5 (LQ–UQ=0.1–13.5) for control patients, in contrast to 0% for the controls (LQ–UQ=0–1.0%), p=0.001). Figure 3 shows that esophageal bile monitoring in patients with Barrett's esophagus and healthy controls varied during the total measuring and supine periods.

The receiver operating curve, plotting the true positive rate (patients with Barrett's esophagus identified by bilirubin exposure) in function of the false positive rate (healthy controls with high bilirubin exposure) is shown in Fig. 4. With an area under the curve of 0.78 (95% CI=0.56–0.89), the ROC analysis of biliary monitoring showed the optimal value for patients at 1% of the TMT [75% sensitivity (95% CI=53–90%), 84% specificity (95% CI=60–96%)]. Therefore, the cutoff value to distinguish normal vs pathologic biliary reflux using 24-h biliary monitoring in the esophagus (absorbance threshold >0.25) should be fixed at 1% of TMT.

Barrett patients, 18 of 24 (75%), 15 of 21 control patients with esophagitis (71%), and 3 of 19 controls (16%) showed biliry reflux into the esophagus more than 1.1% of the TMT (p<0.001). Using this cutoff value, none of the controls, 10 of the control patients (48%), and 11 of 24 Barrett's patients (46%) had pathologic bilirubin exposure during sleep.

Discussion

The results of our study confirm that patients with Barrett's esophagus have significantly more frequent duodenogastric reflux into the esophagus than age- and sex-matched healthy controls. In addition, this reflux, measured by acid and bilirubin exposure, remains longer in the esophagus, especially during sleep.

The role of acid and nonacid reflux into the esophagus as a causative factor of symptoms and mucosal lesions has been addressed in a number of studies. Not only the

Table 2 Median of Acidic Gastric Reflux into the Esophagus in Patients with BM or with Esophagitis and in Healthy Controls

Parameters	Patients with Barrett's Esophagus (n=24) [median (LQ-UQ)]	Patients with Esophagitis (n=21) [median (LQ-UQ)]	Controls (n=19) [median (LQ-UQ)]	Significance Pat. with Barrett vs Controls
Percentage of total measuring time pH<4 (%)	10.6 (6.2–38.3)	19.9 (1.6–71.7)	3.2 (0.9–5.5)	p=0.01
Percentage of upright measuring time pH<4 (%)	11.7 (6.03–6.4)	18.9 (8.7–60.8)	2.4 (0.9–6.1)	p<0.05
Percentage of supine measuring time pH<4 (%)	10.9 (0.4–27.1)	6.3 (0.0–13.3)	0.3 (0.0–4.2)	p=0.004

LQ Lower quartile, *UQ* upper quartile

Table 3 Results of 24-H Intragastric pH and Bile Monitoring in Patients with Barrett Esophagus and Healthy Controls

Parameters	Patients (n=24) [Median (LQ–UQ)]	Controls (n=19) [Median (LQ–UQ)]	Significance
Median of intragastric pH during TMT	1.3 (1.0–1.4)	1.4 (1.1–1.7)	n.s.
Bilirubin exposure percentage (%) of TMT	7.8 (1.6–17.8)	0.0 (0.0–1.0)	p=0.001
Bilirubin exposure percentage (%) of upright time	6.9 (0.1–12.9)	0.0 (0.0–1.3)	p<0.01
Bilirubin exposure percentage (%) of supine time	2.0 (0.0–28.6)	0.0 (0.0–0.0)	p=0.001

LQ Lower quartile, *UQ* upper quartile, *TMT* total measuring time

duration, but possibly the composition of the reflux, is instrumental in the development of such lesions.[13] Twenty-four-hour intragastric bile monitoring has provided the clinician with unequivocal evidence of excessive duodenogastric reflux (DGR) in 41% of patients with endoscopic esophagitis, gastroesophageal reflux (GER) symptoms, and gastric symptoms suggestive of DGR.[19] Reflux of duodenal contents into the stomach, especially postprandially, is a physiological event;[20] however, biliary reflux is a large contributor to mucosal lesions in the whole stomach.[21]

In our study, the control group of patients with different grades of esophagitis showed no significantly different measurements of acidic or bile reflux into the stomach or the esophagus compared to Barrett's patients. This may be caused by selection of patients with esophagitis, which were candidates for fundoplication, but both groups of patients differed significantly compared to healthy controls.

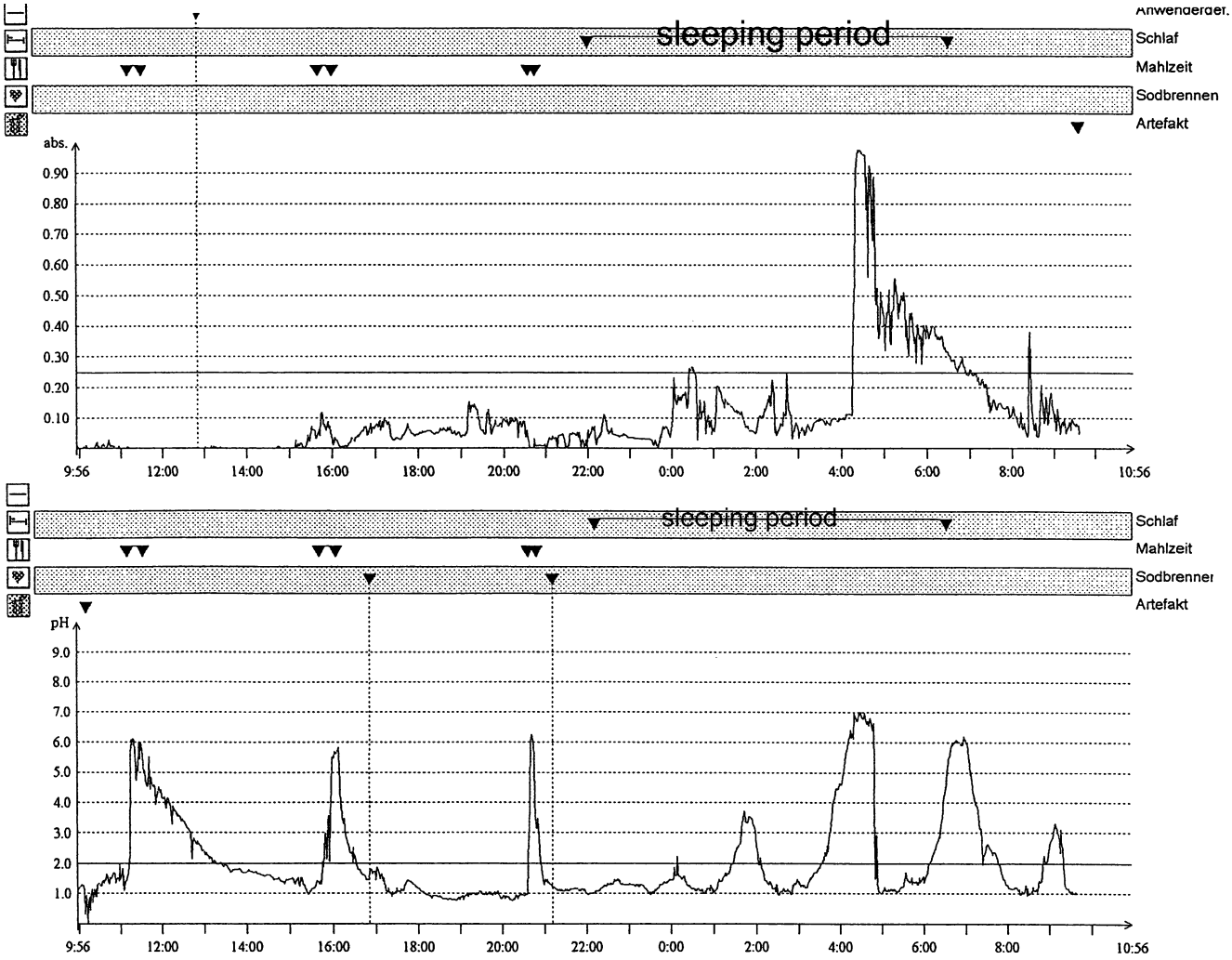

Figure 2 24h intragastric pH- and bile monitoring in a patient with Barrett's esophagus demonstrating the duodenogastric reflux in the early morning. a. Bilitec®-monitoring, b. pH-monitoring.

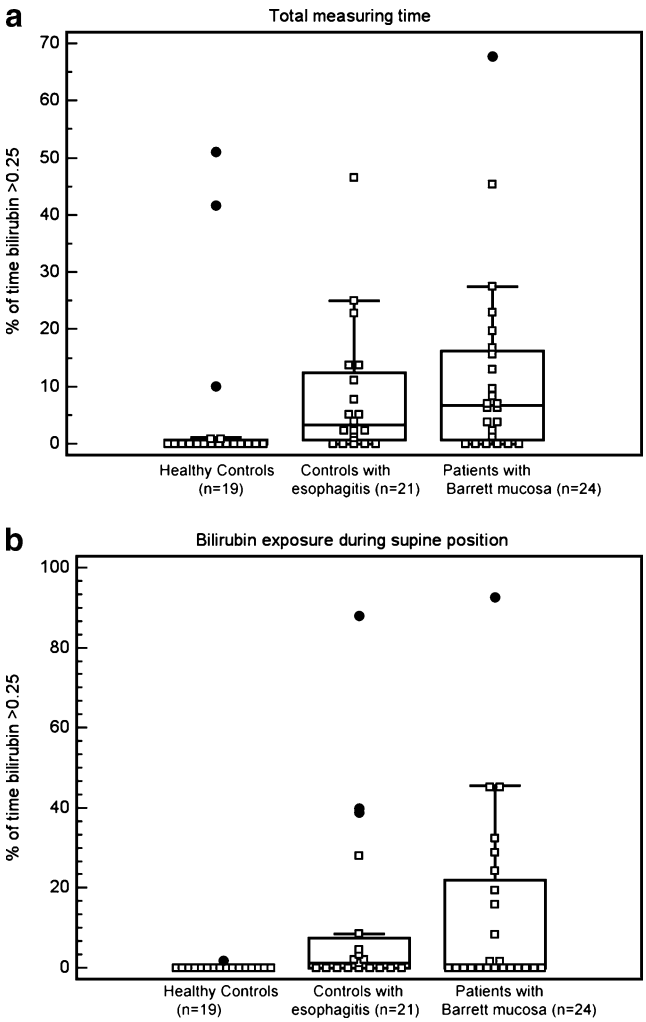

Figure 3 Results of the esophageal bile-monitoring in 24 patients with Barrett's esophagus, 21 patients with esophagitis and 19 healthy controls a) total measuring period (Kruskal-Wallis Test=0.01) b) supine period (Kruskal-Wallis Test p=0.01).

Therefore, our results are of great clinical relevance especially for preoperative diagnostic.

Marshall et al. compared healthy controls to patients with different grades of reflux-esophagitis and Barrett's esophagus with regard to bile measurements in the stomach.[22] In this study, the average age of the control patients was 25 years, and that of the patients in Groups I, II, and III was 42, 50, and 60 years, respectively. The bilitec-probe was positioned 10 cm below the lower esophageal sphincter (LES). The threshold of bilirubin absorbance was 0.14, and although no difference was found between groups over the TMT, gastric bilirubin exposure was higher in the supine than in the upright position. In the current study, the control group was older than that of the Marshall study. More duodeno-gastric reflux was recorded in both study and control patients during all periods of measurement. These findings may be due to improved study conditions.

We used an esophageal threshold of 0.25 for bilirubin absorbance. Fein et al.,[14] in an in vitro study of absorption of different white meals, showed that the least food interference during bile monitoring was measured with an absorbance > 0.25.

Tack et al. reported the influence of meal consistency on Bilitec measurement results in healthy subjects.[23] They compared two groups of young controls. The subjects took either liquid meals only, not absorbing light of the same wavelength as bilirubin, or solid food, avoiding diets that interfere with bilirubin absorbance. The authors found significant differences between the two groups using a bilirubin absorbance threshold >0.14 with a median percentage (interquartile range) over the TMT of 10.9 % (6.7–19.3) for solid meals and 0.3% (0.0–2.8) for liquid meals. Major meal artifacts were present in two-solid-meal (10%) and no-liquid-meal subjects. In our study, we found such a meal artifact in one patient and one control, but the values of bilirubin absorbance were lower than 0.25, and therefore, not relevant to our results.

It is not unusual for gastro-esophageal reflux to contain bile, duodenal, and pancreatic secretions. Utilization of the Bilitec spectrophotometric probe has demonstrated a higher prevalence of abnormal esophageal bilirubin exposure in patients with Barrett's metaplasia when compared to those with erosive injury or without signs of esophagitis.[6,8,10,13] In those studies, patients were consistently older than volunteers included in the control group. However, other studies have shown an increased prevalence of gastro-esophageal reflux with age.[24] For these reasons, we studied

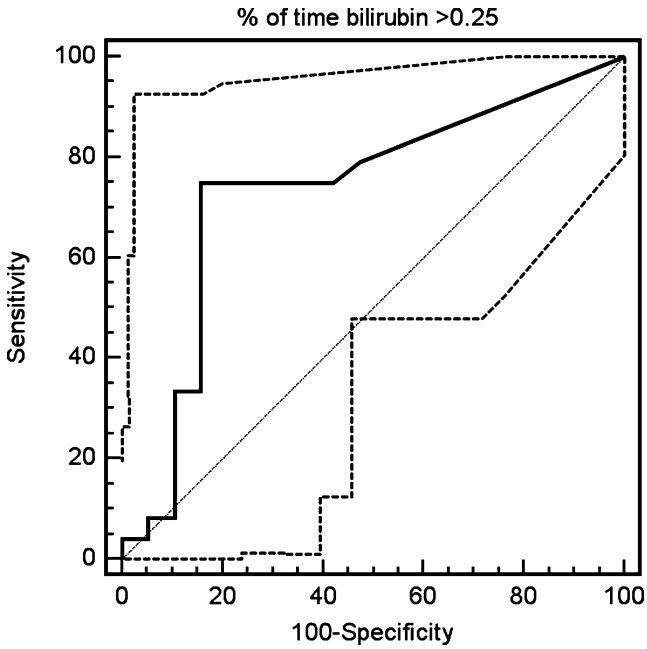

Figure 4 ROC-curve with 95% confidence intervals for pathologic bile-monitoring in patients with Barrett esophagus compared to age and sex matched healthy controls.

age and sex matched healthy controls and patients with Barrett's esophagus or with esophagitis. We found pathologic acid and biliary reflux of the esophagus in one-third of the controls. Perhaps, this may be caused by artefacts or by violation of the protocol by the volunteers. But in a previous published study, we could show that younger healthy controls had no such pathologic reflux.[17] Possibly, these phenomena are caused by relaxations which occur more often in older people. In contrast, nearly all patients with Barrett's esophagus (87%) and all patients with esophagitis (100%) showed pathologic acidic reflux and/or bile reflux measured with combined pH and bile monitoring. Bile reflux into the esophagus during sleep, in particular, was only found in patients with BM or with esophagitis.

In our study, we measured the intragastric pH and the bile reflux from the duodenum into the stomach (DGR). The median of the intragastric pH was similar in both groups. But patients with Barrett's esophagus had significantly longer duodenogastric reflux during the 24-h measuring period than controls. More DGR was demonstrated at night than during the day in both groups of study patients and in healthy controls. This could be associated with an alkaline shift in the pH, according to previously published studies.[25–27] The precise mechanism by which nocturnal DGR occurs and the roles posture plays remain unclear.

Bowrey et al. were unable to establish either gastro-esophageal or duodenogastric reflux as the predominant cause of inflammation in gastric cardiac mucosa with use of the Bilitec 2000 device.[16] This is understandable, as the amount of reflux into the stomach (DGR) does not necessarily correlate with DGER into the esophagus. In this study, the authors demonstrated more DGR in females during the supine period, while males presented more DGER. At the same time, there was no correlation between bile levels in the stomach and esophagus. The controls were, however, much younger than the patients. We found significant differences in bile measurements of the stomach and esophagus between BM patients and controls. In contrast to Bowrey et al., we saw more DGER in females during the supine period and more DGR in male patients.

In contrast, Banki reported similar esophageal exposure to refluxed acid and bilirubin in females and in males with Barrett's mucosa.[28] Pfaffenbach et al.[29] studied esophageal bile and acid reflux in patients with long segment Barrett's esophagus (LSBE), short segment Barrett's esophagus (SSBE) and patients with gastro-esophageal reflux disease (GERD). Subjects underwent esophageal manometry and simultaneous 24-h pH and bile monitoring (Bilitec 2000) with an absorbance value >0.2 for 10.9% of the total period. GER did not differ between the groups. However, DGER differed between patients with LSBE (14.7%), SSBE (2.1%), and GERD (2.1%).

In summary, the analysis of reference values of esophageal acid and bile-reflux measurements in a collective of healthy, age- and gender-matched controls compared to patients with BM led to the following conclusions:

1. Although about 30% of the healthy controls showed acid reflux in pH monitoring, patients with BM had significantly more acid reflux during all measured periods.
2. Healthy controls did not have relevant duodeno-gastric-esophageal reflux measured by bilirubin absorbance. Especially during the supine period, there was no bile reflux.
3. The optimal threshold for pathological bile reflux is 1.1 % (bile monitoring with an absorbance of 0.25).

Acknowledgement The author would like to thank Mrs. Marlis Janson for help with the organization and data measurement of patients and controls.

References

1. Bollschweiler E, Wolfgarten E, Gutschow C, Hoelscher AH. Demographic variations in the rising incidence of esophageal adenocarcinoma in white males. Cancer 2001;92:549–555.
2. Lagergren J, Bergström R, Lindgren A, Nyren O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. N Engl J Med 1999;18:825–831.
3. Wolfgarten E, Rosendahl U, Nowroth T, Leers J, Metzger R, Hoelscher A, Bollschweiler E. Coincidence of nutritional habits and esophageal cancer in Germany. Onkologie 2001;24:546–551.
4. Bollschweiler E, Wolfgarten E, Nowroth T, Rosendahl U, Monig S, Hoelscher A. Vitamin intake and risk of subtypes of esophageal cancer in Germany. J Cancer Res Clin Oncol 2002;128:575–580.
5. Sato T, Miwa K, Sahara H, Hattori T. The sequential model of Barrett's esophagus and adenocarcinoma induced by duodeno-esophageal reflux without exogenous carcinogens. Anticancer Res 2002;22(1A):39–44.
6. Vaezi MF, Richter JE. Duodenogastroesophageal reflux and methods to monitor nonacidic reflux. Am J Med 2001;3(111 Suppl. 8A):160S–168S.
7. Bremner CG, Mason RJ. "Bile" in the esophagus. Br J Surg 1993;80:1374–1376.
8. Stein HJ, Feussner H, Kauer W, DeMeester TR, Siewert JR. Alkaline gastroesophageal reflux: assessment by ambulatory esophageal aspiration and pH monitoring. Am J Surg 1994;167 (1):163–168.
9. Todd JA, Basu KK, De Caestecker JS. Normalization of oesophageal pH does not guarantee control of duodenogastro-oesophageal reflux in Barrett's oesophagus. Aliment Pharmacol Ther 2005;21:969–975.
10. Kauer WK, Peters JH, DeMeester TR, Ireland AP, Bremner CG, Hagen JA. Mixed reflux of gastric and duodenal juices is more harmful to the esophagus than gastric juice alone. The need for surgical therapy re-emphasized. Ann Surg 1995;222(4):525–531.
11. Caldwell MT, Lawlor P, Byrne PJ, Walsh TN, Hennessy TP. Ambulatory oesophageal bile reflux monitoring in Barrett's oesophagus. Br J Surg 1995;82:657–660.
12. Champion G, Richter JE, Vaezi MF, Singh S, Alexander R. Duodenogastro-esophageal reflux: relationship to pH and impor-

tance in Barrett's esophagus. Gastroenterology 1994;107(3):747–754.
13. Vaezi MF, Lacamera RG, Richter JE. Validation studies of Bilitec 2000: an ambulatory duodenogastric reflux monitoring system. Am J Physiol 1994;267(6 Pt 1):G1050–G1057.
14. Fein M, Fuchs KH, Bohrer T, Freys SM, Rocca F. Fiberoptic technique for 24-hour bile reflux monitoring. Dig Dis Sci 1996;1:216–220.
15. Byrne JP, Romagnoli R, Bechi P, Attwood SE, Fuchs KH, Collard JM. Duodenogastric reflux of bile in health: the normal range. Physiol Meas 1999;20(2):149–158.
16. Bowrey DJ, Williams GT, Carey PD, Clark GWB. Inflammation at the cardio-oesophageal junction: relationship to acid and bile exposure. Eur J Gastroenterol Hepatol 2003;15:49–54.
17. Bollschweiler E, Wolfgarten E, Puetz B, Gutschow C, Hölscher AH. Bile reflux into the stomach and the esophagus for volunteers older than 40Â years. Digestion 2005;71(2):65–71.
18. Romagnoli R, Collard JM, Bechi P, Salizzoni M. Gastric symptoms and duodenogastric reflux in patients referred for gatsroesophageal reflux symptoms and endoscopic esophagitis. Surgery 1999;5:480–486.
19. Zweig M, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993;39:561–577.
20. Sharma P, Sampliner R. GERD, DGER, or both in Barrett's esophagus? Am J Gastroenterol 1997;92(5):903–904.
21. Chen SL, Mo JZ, Cao ZJ, Chen XY, Xiao AD. Effects of bile reflux on gastric mucosal lesions in patients with dyspepsia or chronic gastritis. World J Gastroenterol 2005;11(18):2834–2837.
22. Marshall RE, Anggiansah A, Owen WA, Manifold DK, Owen WJ. The extent of duodenogastric reflux in gastro-oesophageal reflux disease. Eur J Gastroenterol Hepatol 2001;13:5–10.
23. Achem AC, Achem SR, Stark ME, DeVault KR. Failure of esophageal peristalsis in older patients: association with esophageal acid exposure. Am J Gastroenterol 2003;98(1): 35–39.
24. Tack J, Bisshops R, Koek G, Sifrim D, Lerut T, Janssens J. Dietary restrictions during ambulatory monitoring of dudenogastroesophageal reflux. Dig Dis Sci 2003;48:1213–1220.
25. Fein M, Fuchs KH, Bohrer T, Freys SM, Thiede A. Fiberoptic technique for 24-hour bile reflux monitoring. Standards and normal values for gastric monitoring. Dig Dis Sci 1996;41(1), 216–225.
26. Thomas H, Wilhelm L, Petermann J, Rosenbaum KD, Lorenz D. Simultaneous long-term measurement of duodenogastric reflux and gastroduodenal motility. Chirurg 1997;68(6):618–623.
27. Hölscher AH, Bollschweiler E, Bumm R. pH-Metrie von Oesophagus und Magen. In Classen M, Siewert JR, Blum AL, eds. Aktuelle Gastroenterologische Diagnostik, 2. Auflage, Hrsg. Berlin Heidelberg New York: Springer, 1992.
28. Banki F, DeMeester SR, Mason RJ, Campos G, Hagen JA, Peters JH, Bremner CG, DeMeester TR. Barrett's esophagus in females: a comparative analysis of risk factors in females and males. Am J Gastroenterol 2005;100:560–567.
29. Pfaffenbach B, Hullerum J, Orth KH, Langer M, Stabenow-Lohbauer U, Lux G. Bile acid reflux in long and short segment Barrett's esophagus, and in reflux disease. Z Gastroenterol 2000;38:565–570.