PAPERS

Diagnostic value of histology in non-erosive gastro-oesophageal reflux disease

N E Schindlbeck, B Wiebecke, A G Klauser, W A Voderholzer, S A Müller-Lissner

Abstract

***Background*—In the absence of oesophageal erosions longterm pH monitoring is the present gold standard for diagnosing gastro-oesophageal reflux disease (GORD). This method, however, is invasive, time consuming, expensive, and not generally available.**

***Aims*—As histological changes have been described in GORD, this study looked at the possibility of whether the diagnosis of non-erosive reflux disease could be made by histological examination routinely during endoscopy.**

***Subjects*—A total of 24 prospectively selected patients with symptoms suggestive of GORD and seven healthy volunteers.**

***Methods*—Oesophageal erosions and other peptic lesions were excluded by oesophagogastroduodenoscopy. Oesophageal pinch biopsy specimens were taken 2 cm and 5 cm above the oesophagogastric junction and evaluated blindly for the histological parameters cellular infiltration, basal zone hyperplasia, and papillary length. Twenty four hour pH monitoring was used as gold standard for the definition of reflux disease. It was abnormal in 13 patients (reflux patients) and normal in 11 patients (symptomatic controls) and in seven healthy volunteers.**

***Results*—Sparse infiltration of the epithelium with lymphocytes in at least one biopsy specimen was found in all patients and volunteers, with neutrophils in three reflux patients, and with eosinophils in two reflux patients and in two healthy volunteers. The basal zone thickness was increased in three reflux patients, in one symptomatic control, and in one healthy volunteer. The papillary length was greater than two thirds of total epithelium in six of 13 reflux patients in contrast with none in 11 symptomatic controls ($p<0.05$) and to one healthy volunteer. The sensitivity of the parameter papillary length hence was only 46%.**

***Conclusions*—Although gastro-oesophageal reflux produces slight histological changes apart from oesophageal erosions in a few subjects, none of the established histological parameters can fulfil the standards of a diagnostic tool. Routine pinch biopsies can not be recommended for the diagnosis of GORD in patients without visible oesophageal erosions.**

(*Gut* 1996; **39:** 151–154)

Keywords: gastro-oesophageal reflux disease, oesophageal pH monitoring, histology, oesophagus, endoscopy.

Medizinische Klinik
N E Schindlbeck
A G Klauser
W A Voderholzer
S A Müller-Lissner

and Pathologisches Institut
B Wiebecke

Klinikum Innenstadt, University of Munich, Germany

Correspondence to:
Dr N E Schindlbeck, Medizinische Klinik, Klinikum Innenstadt der Universität, Ziemssenstrasse 1, D-80336 Munich, Germany.

Accepted for publication 27 March 1996

Non-erosive gastro-oesophageal reflux disease (GORD) is the most probable diagnosis in dyspeptic patients when organic diseases, such as peptic ulcers, oesophageal erosions, or carcinomas, have been excluded by oesophagogastroduodenoscopy.[1–4] In these patients 24 hour pH monitoring is a highly sensitive and specific test for diagnosing reflux disease.[5–7] This method, however, is invasive, time consuming, expensive, and not generally available in practice.

As histological abnormalities have been described in GORD,[8 9] it seems rational to diagnose non-erosive reflux disease by simple oesophageal pinch biopsies during oesophagogastroduodenoscopy. These mild histological changes are mainly infiltration with neutrophils and eosinophils, elongated papillae, and basal zone thickening.[9] However, the available data on the diagnostic value of these histological criteria are contradictory.[10–13] This study, therefore, was performed to prospectively evaluate, whether GORD can be diagnosed by histological evaluation of oesophageal biopsy specimens in well defined reflux patients without oesophageal erosions and in adequate controls.

Methods

Patients

We prospectively included 24 patients (13 men, 11 women, mean age 45·9 years, range 20–70) referred to our outpatient department for evaluation of symptoms compatible with GORD, namely heartburn, acid regurgitation, epigastric pain, or other less specific symptoms.[14] For each patient a standardised questionnaire was completed during a personal interview by an experienced gastroenterologist asking for type, frequency, and duration of symptoms. Patients with suspected or confirmed coronary artery disease or with previous upper gastrointestinal surgery such as cholecystectomy, gastric resection, or

TABLE I *Reflux parameters in patients and volunteers*

	Reflux time upright %	*Reflux time supine %*	*Mean acidity (mmol/l)*
Reflux patients (n=13)	10·4 (5·7–27·3)*	3·7 (0·01–15·8)*	0·41 (0·065–8·45)*
Symptomatic controls (n=11)	2·5 (0·65–7·9)	0·24 (0·0–2·84)	0·031 (0·0–0·16)
Healthy volunteers (n=7)	4·0 (0·24–7·5)	1·2 (0·0–2·4)	0·05 (0·0–0·19)

Median (range). *=p<0·05 versus symptomatic controls and healthy volunteers (Mann-Whitney U test).

TABLE II *Cellular infiltration of the epithelium*

	Site of biopsy above lower oesophageal sphincter	
	2 cm	*5 cm*
	neutrophils/ eosinophils	*neutrophils/ eosinophils*
Reflux patients (n=13)	3/2	0/0
Symptomatic controls (n=11)	0/0	0/0
Healthy volunteers (n=7)	0/2	0/0

Number of patients with neutrophils/eosinophils is shown.

selective proximal vagotomy were excluded.

GORD was defined by the result of 24 hour pH monitoring (see below). Thirteen patients had abnormal results during pH monitoring (reflux patients). The remaining 11 patients had normal pH monitoring (symptomatic controls).

Volunteers

The seven healthy volunteers (five men, two women, mean age 25·1 years, range 20–32) were completely free of even occasional reflux symptoms. All volunteers had normal results during pH monitoring.

Endoscopy and histology

Oesophagogastroduodenoscopy was performed in all subjects using Olympus fibrescopes with a 2·8 mm channel. Subjects with any visible lesions, particularly oesophageal erosions and ulcers, were excluded. Oesophageal biopsy specimens were taken 2 cm and 5 cm above the oesophagogastric junction with Olympus biopsy forceps. These biopsy sites were selected in accordance to the literature.[8–13 15] Two biopsy specimens were taken from each site. To obtain sufficient material and to ensure almost vertical pinch biopsy specimens the opened forceps was withdrawn towards the tip of the scope and the scope was bent upwards maximally and hence the forceps was pressed vertically against the oesophageal wall. Specimens were carefully mounted on a filter paper and fixed in 10% formalin. The specimens were visually oriented with slight magnification during embedding in the paraffin wax blocks before hardening to ensure vertical cutting. They were cut and finally stained with haematoxylin and eosin. The histological evaluation of the specimens was performed in a blinded manner. The pathologist (BW) was neither aware, whether the subject was a patient or a volunteer, nor did he know the result of pH monitoring. A slight superficial damage of the epithelium was assumed if the cell connections of the upper surface of the epithelium were not intact, fragile, or otherwise damaged. The histological parameters basal zone hyperplasia (normally ≤15% of total epithelium), papillary length (normally ≤66%), and semiquantitative cellular infiltration with lymphocytes, neutrophils, and eosinophils were evaluated.[8 9 15] An ocular micrometer was used to determine papillary length and basal zone thickness.[9 15] The thickness of the basal zone was defined according to Mitros[15] from the basement membrane to that point, at which the nuclei of the squamous cells are separated from each other by more than one nuclear diameter. Our methods provided sufficiently interpretable pinch biopsy specimens in the included patients and volunteers. Two further patients were not included because of insufficient material or wrong orientation of the specimens.

Twenty four hour pH monitoring

Ambulatory oesophageal 24 hour pH monitoring was performed with combined glass electrodes (440 M4, Ingold, Urdorf Switzerland) in combination with portable recorders (Autronicord CM 24 pH, Autronic, Germany/ Flexilog 2000, Oakfield Instruments, Eynsham, Witney Oxon, United Kingdom) as previously described.[7 16 17] Drugs affecting gastrointestinal secretion or motility were stopped for at least 36 hours before pH monitoring. No patient had omeprazole at least seven days before inclusion. During pH monitoring each patient completed a standardised diary recording times and types of meals, periods of upright and supine body position, and time, duration, and type of symptoms. After 24 hours the electrode was removed and the stored data were evaluated by computer analysis. In addition, they were edited on an analogue writer for visual quality control.

The reflux parameters percentage time with oesophageal pH less than 4 and mean acidity, the mean of all measured H^+ concentrations (antilog pH), were calculated.[5–7 18 19] A subject was considered to have a normal result during pH monitoring, if the percentage reflux time was <8·2% for the upright and <3·0% for the supine body position, respectively. These thresholds have been established in larger samples previously.[7 18 19]

Statistics

Data are given as means, medians, and ranges. The Mann-Whitney U test, the χ^2 test, and the Fisher test were used to test for statistically significant differences.

The study protocol had been approved by the local ethical committee. All patients and volunteers had given their consent.

Results

Table I summarises the reflux parameters of pH monitoring. As expected, reflux patients, defined by abnormal pH monitoring, had significantly more acid reflux compared with symptomatic controls with normal result during pH monitoring and healthy volunteers.

A slight superficial damage of the oesophageal mucosa in at least one biopsy

TABLE III *Basal zone thickness (% of total epithelial thickness)*

	Site of biopsy above lower oesophageal sphincter					
	2 cm			*5 cm*		
Basal zone thickness	*≤15%*	*16–30%*	*30–50%*	*≤15%*	*16–30%*	*30–50%*
Reflux patients (n=13)	11	1	1	10	3	0
Symptomatic controls (n=11)	11	0	0	10	1	0
Healthy volunteers (n=7)	6	1	0	7	0	0

Number of patients is shown.

TABLE IV *Papillary length (% of total epithelial thickness)*

	Site of biopsy above lower oesophageal sphincter			
	2 cm		*5 cm*	
Papillary length	*≤66%*	*>66%*	*≤66%*	*<66%*
Reflux patients (n=13)	7	6*	12	1
Symptomatic controls (n=11)	11	0	10	1
Healthy volunteers (n=7)	6	1	6	1

Number of patients is shown, *=$p<0.05$ versus symptomatic controls (χ^2 test).

specimen was found in all healthy volunteers, in nine of 13 reflux patients, and in eight of 11 symptomatic controls (no significant differences). As a differentiation between mechanical artefacts and acid related damage is not possible, this superficial damage is useless for diagnosing gastro-oesophageal reflux disease. A slight lymphocytic infiltration of the epithelium in at least one biopsy specimen was found in all patients and volunteers, irrespective of the site where the specimen was taken. Infiltration of the epithelium with neutrophils was present in only three reflux patients and with eosinophils in two reflux patients and in two healthy volunteers (Table II).

The basal zone thickness was normal in most patients and volunteers. It was slightly increased in three reflux patients, in one symptomatic control, and in one healthy volunteer (Table III). The papillary length was greater than two thirds of total epithelium in six of 13 reflux patients in contrast with none in 11 symptomatic controls (Table IV). Although the difference was significant ($p<0.05$), the sensitivity of the parameter papillary length for the diagnosis of non-erosive reflux disease reached only 46%.

Discussion

GORD is a frequent cause of dyspepsia with a prevalence of up to 8% in the community.[2 4 20] Endoscopy of the upper gastrointestinal tract is the most common diagnostic procedure in these patients. Reflux disease is proved if characteristic erosions[21] are macroscopically visible in the distal oesophagus. Oesophageal erosions, however, are present only in up to one third of patients with symptomatic reflux.[2 3 7 22] Macroscopic findings during endoscopy for the diagnosis of reflux disease, therefore, are hampered by a low sensitivity.

As histological changes, such as basal cell hyperplasia and location of the papillae close to the epithelial surface, have been described in GORD,[8,9] it seemed rational to increase the sensitivity of endoscopy in non-erosive reflux disease by histological examination. A frequently quoted study on histological parameters in reflux oesophagitis included 33 reflux patients with severe heartburn and 19 miscellaneous patients.[8] They were partly classified with respect to reflux disease by a modified acid perfusion test and other provocative manoeuvers, which have been shown to be inferior to pH monitoring with respect to diagnostic accuracy.[5 7 17] Oesophagoscopy, which was performed in 34 patients, showed macroscopic evidence of reflux oesophagitis, as defined by ulcerations, friability, or granularity of the oesophageal mucosa, in 25 patients. It is not surprising that a good correlation between macroscopic oesophagitis and histological parameters was reported. Another study[11] found an agreement between macroscopic and histological findings in the oesophagus in about 50% of the included patients. In patients without macroscopic abnormalities, however, the correlation between histological findings and reflux disease, as defined by symptoms, was rather poor. It has been clearly shown, that the diagnosis of reflux disease usually is no problem in patients with typical symptoms or oesophageal erosions.[6 14]

The most important requirement for a reliable diagnostic test is a high sensitivity and specificity with respect to an appropriate gold standard. For diagnosing gastro-oesophageal reflux disease the gold standard is at present longterm pH monitoring. This method, therefore, was used to define reflux disease in the absence of oesophageal erosions in our study. We did not use symptom indices, because they measure the individual sensitivity of oesophageal mucosa to acid, which does not necessarily correlate with the quantity of acid reflux. In contrast, histological changes of oesophageal mucosa have to be assumed to depend on the quantity of reflux.

Our data cannot support the view that histological parameters are a good diagnostic tool for non-erosive reflux disease. A slight lymphocytic infiltration of the epithelium was common in both patients and volunteers, which is in accordance with the literature.[9 15] Infiltration with neutrophils and eosinophils and basal zone thickening could only be shown in a few of our reflux patients and were also seen in some healthy volunteers. The sensitivity of these parameters, therefore, proved to be very low. In addition they were by no means highly specific. A significant difference between patients with abnormal and normal pH monitoring was found only for the parameter papillary length greater than 66% of total epithelium. The corresponding sensitivity of 46%, however, shows that this parameter is far from being a useful diagnostic criterion. Comparable results were found in some prior investigations.[12 13] A more recent study[12] in well defined patient groups with respect to presence or absence of macroscopic findings and the result of pH monitoring found that neither histology nor morphometry could be

recommended as a diagnostic tool for non-erosive reflux disease. Thus, the initial optimism regarding the diagnosis of non-erosive reflux disease by histology has been disproved.

Nevertheless, pinch biopsies of the distal oesophagus and the oesophagogastric junction are necessary in some circumstances, for example, for the differential diagnosis of macroscopic abnormalities of the oesophageal mucosa not characteristic for gastro-oesophageal reflux. As the prevalence of Barrett's oesophagus and adenocarcinoma of the oesophagogastric junction are rising,[23–26] routine pinch biopsies of the oesophagogastric junction can be recommended to detect metaplasia and early carcinoma in patients with highly suspected or proved reflux disease.

In conclusion, gastro-oesophageal reflux is able to produce histological changes apart from oesophageal erosions in a minor proportion of patients with non-erosive reflux disease. These are the well known histological parameters infiltration with neutrophils and eosinophils, basal zone hyperplasia, and elongation of the papillae. The critical review of our data and the available published studies, however, failed to show that any of these parameters can fulfil the standards of a sufficient diagnostic tool. Routine pinch biopsies, therefore, cannot be recommended for the diagnosis of GORD.

1 Colin-Jones DG, Bloom B, Bodemar G, Crean G, Freston J, Gugler R, *et al.* Management of dyspepsia: report of a working party. *Lancet* 1988;i:576–9.
2 Heading RC. Epidemiology of oesophageal reflux disease. *Scand J Gastroenterol* 1989; **24** (suppl 168): 33–7.
3 Klauser AG, Voderholzer WA, Knesewitsch PA, Schindlbeck NE, Müller-Lissner SA. What is behind dyspepsia? *Dig Dis Sci* 1993; **38:** 147–54.
4 Nebel OT, Fornes MF, Cattell DO. Symptomatic gastro-esophageal reflux: incidence and precipitating factors. *Dig Dis Sci* 1976; **21:** 953–6.
5 DeMeester TR, Wang C-I, Wernly JA, Pellegrini CA, Little AG, Klementschitsch P, *et al.* Technique, indications, and clinical use of 24 hour esophageal pH monitoring. *J Thorac Cardiovasc Surg* 1980; **79:** 656–70.
6 Klauser AG, Heinrich C, Schindlbeck NE, Müller-Lissner SA. Is long-term esophageal pH monitoring of clinical value? *Am J Gastroenterol* 1989; **84:** 362–6.
7 Schindlbeck NE, Heinrich Ch, König A, Dendorfer A, Pace F, Müller-Lissner SA. Optimal thresholds, sensitivity and specificity of long-term pH-metry for the detection of gastroesophageal reflux disease. *Gastroenterology* 1987; **93:** 85–90.
8 Ismail-Beigi F, Horton PF, Pope CE. Histological consequences of gastroesophageal reflux in man. *Gastroenterology* 1970; **58:** 163–74.
9 Frierson HF. Histology in the diagnosis of reflux esophagitis. *Gastroenterol Clin North Am* 1990; **19:** 631–44.
10 Knuff TE, Benjamin SB, Worsham GF, Hancock JE, Castell DO. Histologic evaluation of chronic gastro-esophageal reflux. *Dig Dis Sci* 1984; **29:** 194–201.
11 Funch-Jensen P, Kock K, Christensen LA, Fallingborg J, Kjaergaard JJ, Andersen SP, *et al.* Microscopic appearance of the esophageal mucosa in a consecutive series of patients submitted to upper endoscopy. *Scand J Gastroenterol* 1986; **21:** 65–9.
12 Collins JSA, Watt PCH, Hamilton PW, Collins BJ, Sloan JM, Elliot H, *et al.* Assessment of oesophagitis by histology and morphometry. *Histopathology* 1989; **14:** 381–9.
13 Seefeld U, Krejs GJ, Siebenmann RE, Blum AL. Esophageal histology in gastroesophageal reflux. Morphometric findings in suction biopsies. *Dig Dis Sci* 1977; **22:** 956–64.
14 Klauser AG, Schindlbeck NE, Müller-Lissner SA. Symptoms in gastro-oesophageal reflux disease. *Lancet* 1990; **335:** 205–8.
15 Mitros FA. Atlas of gastrointestinal pathology. Philadelphia: Lippincott, 1988:1.2–1.12.
16 Klauser AG, Schindlbeck NE, Müller-Lissner SA. Esophageal 24-h pH monitoring: is prior manometry necessary for correct positioning of the electrode? *Am J Gastroenterol* 1990; **85:** 1463–7.
17 Emde C, Garner A, Blum AL. Technical aspects of intraluminal pH-metry in man: current status and recommendations. *Gut* 1987; **28:** 1177–88.
18 Schindlbeck NE, Ippisch H, Klauser AG, Müller-Lissner SA. Which pH-threshold is best in esophageal pH-monitoring. *Am J Gastroenterol* 1991; **86:** 1138–41.
19 Schindlbeck NE, Klauser AG, Voderholzer WA, Müller-Lissner SA. Mean acidity or percentage reflux time in oesophageal pH-monitoring? *Eur J Gastroenterol Hepatol* 1993; **5:** 155–9.
20 Wienbeck M, Barnert J. Epidemiology of reflux disease and reflux esophagatis. *Scand J Gastroenterol* 1989; **24** (suppl 156): 7–13.
21 Savary M, Miller G. *The Oesophagus.* Solothurn: Gassmann, 1977.
22 Schindlbeck NE, Klauser AG, Berghammer G, Londong W, Müller-Lissner SA. Three year follow up of patients with gastrooesophageal reflux disease. *Gut* 1992; **33:** 1016–9.
23 Cameron AJ. Epidemiologic studies and the development of Barrett's esophagus. *Endoscopy* 1993; **25** (suppl): 635–6.
24 Spechler SJ. Epidemiology and natural history of gastro-oesophageal reflux disease. *Digestion* 1992; **51** (suppl 1): 24–9.
25 Spechler SJ, Zeroogian JM, Antonioli DA, Wang HH, Goyal RK. Prevalence of metaplasia at the gastro-oesophageal junction. *Lancet* 1994; **344:** 1533–6.
26 Pera M, Cameron AJ, Trastek VF, Carpenter HA, Zinsmeister AR. Increasing incidence of adenocarcinoma of the esophagus and esophagogastric junction. *Gastroenterology* 1993; **104:** 510–3.