Diploma Thesis

Evolving Patterns in the Histologic Diagnosis of Reflux Esophagitis

submitted by

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conducted at the

Institute of Pathology

under supervision of

Univ. Doz. Dr. Cord Langner

Graz, May 2014 Nora Schneider

Affidavit

I hereby declare that the following diploma thesis has been written by me and without any assistance from third parties. For the preparation of this thesis I have not used any other sources than those indicated sources in the thesis itself.

Please note that this thesis has already been published in parts in the peer-reviewed journals “Human Pathology” and “Histopathology”:

Schneider NI et al. *Validation study of the Esohisto consensus guidelines for the recognition of microscopic esophagitis (histoGERD Trial).* Hum Pathol. 2014 May;45:994-1002. Epub 2014 Jan 17.

Langner C, Schneider NI et al. *Cardiac mucosa at the Gastroesophageal Junction: Indicator of Gastroesophageal Reflux Disease? Data from a Prospective Central European Multicenter Study on histologic and endoscopic diagnosis of esophagitis (histoGERD Trial).* Histopathology. 2014 Jan 7. [Epub ahead of print]

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Die Ergebnisse dieser Arbeit wurden bereits teilweise in den Zeitschriften „Human Pathology“ und „Histopathology“ veröffentlicht:

Schneider NI et al. *Validation study of the Esohisto consensus guidelines for the recognition of microscopic esophagitis (histoGERD Trial).* Hum Pathol. 2014 May;45:994-1002. Epub 2014 Jan 17.

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Graz, Mai 2014 Nora Schneider

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Abstract

Objectives: Gastroesophageal reflux disease is a common disease with a rising prevalence in the world. In patients with gastroesophageal reflux disease histology is generally believed to be a tool of limited diagnostic value.

Our study aimed to assess the prevalence and evaluate the clinical significance of microscopic esophageal lesions as defined by the Esohisto consensus guidelines which have proven high interobserver agreement in previous studies.

Methods: In the prospective Central European multicenter *histo*GERD trial we recruited 1,071 individuals (576 female, 495 males; median age 53 years, range 15-93) undergoing gastroscopy for non-selected reasons. Biopsy material was systematically sampled from above and below the gastroesophageal junction. To validate the clinical importance of the histologic Esohisto criteria, we related their presence to clinical data including endoscopic findings as well as patients´ symptoms and therapy of gastroesophageal reflux disease.

Results: Overall, histologic diagnosis of mild and severe esophagitis was made in 423 (39.5%) and 296 (27.6%) individuals, respectively, while the squamous mucosa of 352 (32.9%) individuals was normal upon histology or showed only insignificant findings. Proliferative changes of the squamous epithelium, in particular basal cell layer hyperplasia, papillary elongation, and intercellular space dilation were more common than inflammatory cell infiltration. The presence of microscopic esophagitis was associated with male gender ($p=0.009$), patients' symptoms ($p=0.003$), history of proton pump inhibitor intake ($p<0.001$), and the endoscopic diagnosis of esophagitis ($p<0.001$). Notably, among the 450 patients with no endoscopic signs of esophagitis (Los Angeles Category N), 41.8% were identified with mild, and 17.1% with severe (microscopic) esophagitis, respectively, indicating higher sensitivity of histologic diagnosis.

Conclusions: Our data illustrate the value of histology in the work-up of patients with reflux disease: We suggest that biopsies should routinely be obtained when patients undergo upper gastrointestinal endoscopy for evaluation of gastroesophageal reflux disease and may particularly be beneficial in patients with non-erosive reflux disease.

Zusammenfassung

Hintergrund: Die gastroösophageale Refluxkrankheit ist ein häufiges Krankheitsbild. In der Diagnosestellung der Refluxkrankheit hat die Histologie nur einen geringen Stellenwert. Eine Standardisierung diverser histologischer Kriterien zur Beurteilung und Klassifikation der mikroskopischen Ösophagitis erfolgte im Jahre 2010 im Rahmen des Esohisto-Projekts Unsere Studie zielt auf die Erfassung der Prävalenz dieser histologischen Kriterien sowie deren klinische Validation.

Methoden: In der prospektiven multizentrischen *histo*GERD Studie nahmen 1071 Personen teil (576 Frauen, 495 Männer; Durchschnittsalter 53 Jahre, Altersspanne 15 bis 93 Jahre), welche sich aus unterschiedlichen Gründen gastroskopieren ließen. Mehrere Biopsien wurden nach einem standardisierten Protokoll oberhalb und unterhalb der gastroösophagealen Grenzzone entnommen. Zur klinischen Validierung des Esohisto-Projekts untersuchten wir die Prävalenz der histologischen Parameter sowie deren Zusammenhänge mit klinischer Symptomatik und endoskopischer Diagnose der Refluxösophagitis.

Ergebnisse: Histologisch wurde eine milde Ösophagitis bei 423 (39,5%) PatientInnen diagnostiziert, sowie eine schweren Ösophagitis bei 296 (27,6%) PatientInnen. Keine oder nur wenige Veränderungen zeigte das Plattenepithel des Ösophagus bei 352 (32,9%) TeilnehmerInnen. Unter den histologischen Veränderungen des Plattenepithels waren die proliferativen Parameter des Plattenepithels (Verbreiterung der Basalzelllagen und Verlängerung der Stromapapillen) und dilatierte Interzellularräume häufiger vorhanden als eine Infiltration durch Entzündungszellen. Eine vorhandene mikroskopische Ösophagitis war assoziiert mit männlichem Geschlecht ($p=0,009$), Refluxsymptomen ($p=0,003$), positiver Medikamentenanamnese von Protonenpumpenhemmern ($p<0,001$), und der endoskopischen Diagnose einer Ösophagitis ($p<0,001$).

Schlussfolgerung: Unsere Daten verdeutlichen, dass die histologische Diagnose der Refluxkrankheit durchaus möglich ist. Wir empfehlen die routinemäßige Entnahme von Biopsien in der Diagnosestellung der Refluxkrankheit, da die histologische Untersuchung eine sinnvolle und dabei preisgünstige Ergänzung darstellt, speziell bei PatientInnen mit nichterosiver Refluxkrankheit.

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Abbreviations

CM	Cardiac mucosa
GEJ	Gastroesophageal junction
GERD	Gastroesophageal reflux disease
NERD	Non-erosive reflux disease
OM	Oxyntic mucosa
OCM	Oxyntocardiac mucosa
PPI	Proton pump inhibitor
SCJ	Squamocolumnar junction

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Introduction

Gastroesophageal reflux disease (GERD) is a common disease [1]. The prevalence GERD is rising worldwide, with the highest prevalence in the Western world (Europe 23.7% and USA 28.8%) [1] and increasing prevalence even in Asia (East Asia 2.5-9.4%, Mid Asia 7.6-19.4%, Western Asia 12.5-27.6%) where reflux disease has not traditionally been a major health problem in the past [1, 2]. Patients suffering from more frequent and severe GERD symptoms have a poorer health-related quality of life and a higher impairment in work productivity [3]. Additionally, GERD-related absence from work, numerous medical consultations or hospitalizations result in an economic burden for society [1, 3, 4].

The esophagus is a tubular organ with an overall length of approximately 23-25 cm. It ranges from the cricoid cartilage, through the thorax to its junction with the stomach, located several centimeters past the diagphragm [5].
Upon histology, the esophagus is lined by non-keratinizing, stratified squamous epithelium, and the stomach is lined by mucinous columnar epithelium [5, 6]. Between the stomach and the esophagus is the gastroesophageal junction (GEJ). It contains the lower esophageal sphincter, characterized as a variable zone of distal 2-4 cm and a pressure of approximately 10-26 mmHg, which is above, both intragastric and intraesophageal pressures [5, 6]. Upon endoscopy, the GEJ is the point where the tubular esophagus meets the gastric mucosal folds [6, 7], also known as the “Z-line” [5]. In “normal” individuals, the GEJ is identical with the squamocolumnar junction (SCJ), the histologic transition point between the esophageal squamous epithelium and the gastric mucinous columnar epithelium. In patients with chronic reflux the SCJ moves proximally as a result of metaplasic replacement. Thus, the histologic SCJ is located above the anatomic GEJ [6].

In the setting of prolonged reflux from acid gastric juice into the distal esophagus, inflammation and mucosal breaks of the esophageal squamous epithelium may develop. Subsequently, reduced reparative capacity of esophageal mucosa and a microenviroment of a low pH leads to abnormal differentiation of pluripotent stem cells, located in the basal cell layer, into columnar epithelium (gastric type or intestinal type) [8, 9]. Thus, the squamous epithelium in the distal esophagus is

replaced by metaplastic columnar epithelium that is more resistant to injury from acid gastric content [9, 10].

Typical GERD symptoms are heartburn and regurgitation. As defined by an international consensus group, is heartburn a burning sensation in the retrosternal area and regurgitation is defined as the perception of flow of refluxed gastric content into the mouth or hypopharynx [11]. Additionally, patients with GERD report symptoms such as epigastric pain or night-time heartburn and sleep disturbance [11].Further symptoms and an overview are presented in Figure 1. In clinical practice it is important to differentiate between cardiac chest pain and non-cardiac chest pain, because GERD can cause episodes of chest pain that resemble ischemic cardiac pain (i.e. reflux chest pain syndrome) [11, 12].

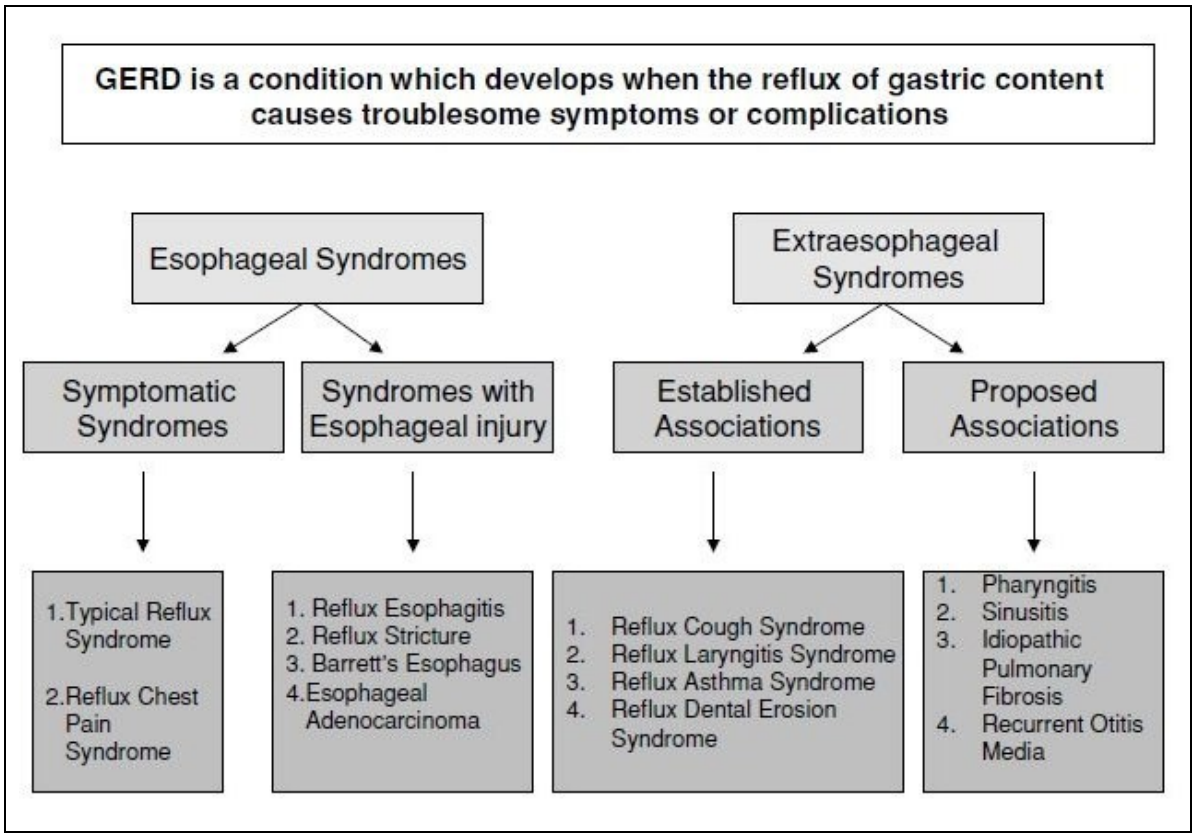


Figure 1: The overall definition of GERD and its constituent symptoms [11].

GERD comprises a large spectrum of clinical manifestations [11, 13], including patients with typical symptoms and visibly endoscopic reflux esophagitis or otherwise patients without symptoms of reflux disease and macroscopic visible esophagitis with varying extent of mucosal breaks, graded according to the modified Los Angeles classification [14, 15]. Non-erosive reflux disease (NERD) is characterized by the presence of troublesome reflux symptoms and the absence of mucosal breaks at endoscopy [11] but with histologic changes of the squamous epithelium (i.e. microscopic esophagitis) [11, 13].

According to recently published practice guidelines of the American College of Gastroenterology a presumptive diagnosis of GERD can be made on the basis of typical symptoms [12, 16]. Improvement of reflux symptoms on empiric medical therapy with a proton pump inhibitor (PPI) ideally confirms this symptom-based diagnosis (so-called PPI test) [12, 16]. Even though esophageal pH manometry is the gold standard for detection of increased acid exposure, it is only advised for preoperative evaluation or arguable presentation of GERD. Upper gastrointestinal endoscopy is only recommended in the presence of permanent alarm symptoms and screening of patients at high risk for complications. Thus, gastroscopy, with biopsy taking from the distal esophagus is not required in the presence of typical GERD symptoms in clinical practice [12, 16].

Early diagnosis of GERD is crucial because chronic reflux esophagitis is a key risk factor for the development of Barrett´s esophagus, which is a precursor lesion for esophageal adenocarcinoma [17, 18].
Notably, GERD patients experiencing symptoms of reflux on a regular basis (daily or at least once a week) have an increased risk in developing esophageal adenocarcinoma, although the absolute risk is quite low [19, 20].

Various histologic features of the squamous epithelium related to GERD have been identified. These include the classical parameters for proliferative changes of the squamous epithelium, basal cell layer hyperplasia and papillary elongation [21, 22], as a compensatory result of damage to the esophageal epithelium [23]. Both parameters show good results in the histologic diagnosis of GERD [24]. More recently, dilation of intercellular spaces has been identified as histologic marker

[25] of cellular damage induced by luminal acid reflux. In particular, patients with NERD show scores of dilated intercellular spaces three times higher than healthy controls [22, 26, 27]. Furthermore, dilation of intercellular spaces as a histologic feature has proven good interobserver agreement in the diagnosis of esophagitis [28]. In microscopic esophagitis an increase in inflammatory cells, especially intraepithelial neutrophils [29], eosinophils [30, 31] and mononuclear cells [32, 33] are common but unspecific histologic features in reflux esophagitis [34].
Other authors, investigating different types of columnar epithelium at the GEJ, observed that the presence of cardiac mucosa (CM) and oxyntocardiac mucosa (OCM) at the GEJ is associated with abnormal pH values in the distal esophagus. In addition, the presence of intestinal metaplasia correlates with the length of CM which indicates the severity of GERD [35, 36]. Notably, the point of origin and the significance of CM remains a contentious issue in the literature.

In patients with non-erosive reflux disease (NERD) histology is generally believed to be a tool of limited diagnostic value, due to low sensitivity and specificity [37]. Furthermore, various histologic lesions associated with reflux do exist, but were not specifically observed in patients with GERD [38].
Several studies have, however, demonstrated that histology, if systematically applied, may render important diagnostic clues as at least two thirds of NERD patients have histologic evidence of esophageal injury [22, 39].

The Esohisto project is a multinational initiative for the standardized recognition of microscopic lesions in patients with GERD [13, 40]. Histologic lesions evaluated were basal cell layer hyperplasia, papillary elongation, intraepithelial eosinophil, neutrophil and mononuclear cell number as well as necrosis/erosion, healed erosion, and dilation of intercellular spaces. The project has proven good interobserver agreement (ranging from 64% to 97%).

In addition to evaluating the severity of each histologic criterion, a combined severity score was also developed for each patient to grade the severity of esophagitis. The interobserver agreement for the combined severity score was 77% [40, 41]. However, a validation study, correlating the evaluated microscopic features with clinical variables, such as reflux symptoms and endoscopic features, has not been performed yet.

In the prospective Central European multicenter *histo*GERD trial we recruited 1,130 individuals undergoing upper gastrointestinal endoscopy for various non-selected reasons. Biopsy material was systematically sampled from the esophagus, the GEJ and the stomach. In this study we aimed to assess the prevalence of microscopic esophageal lesions, as defined in the Esohisto consensus guidelines. Specifically, we related their presence to various clinical and endoscopic features indicative of GERD, thereby evaluating the clinical significance of the Esohisto project.

Material and Methods

We conducted a prospective cross-sectional study to assess the prevalence of histologic esophageal lesions related to GERD and to assess the prevalence of different forms of gastritis. My colleague Dr. med. univ. Eva-Maria Wolf was in charge of the evaluation of the biopsies sampled from the stomach and presented her results in a previous diploma thesis [42] and a peer-reviewed journal [43].

In this study, we used the standardized Esohisto consensus guidelines [13] and the combined severity score [40] to assess the prevalence of histologic esophageal lesions related to GERD. To validate the clinical importance of these histologic criteria, we related their presence to clinical data such as endoscopic findings, symptoms and therapy of GERD. Data will be presented following the STROBE Statement aimed at strengthening the reporting of observational studies [44].

Study Population

Participants, undergoing endoscopic examination of their upper gastrointestinal tract were prospectively recruited in the multicenter central European *histo*GERD trial that aimed to systematically investigate clinical data, particularly endoscopic and histological findings in individuals with and without symptoms of reflux esophagitis.

In Austria three clinical departments (Department of Internal Medicine, Krankenhaus der Barmherzigen Brüder, St. Veit/Glan; Department of Surgery, Division of General Surgery, Medical University of Graz; Department of Internal Medicine, Division of Gastroenterology and Hepatology, Medical University of Graz) collaborated in the *histo*GERD trial and participants were recruited between November 2011 and April 2012. In Germany two private practices for gastroenterology (Dr. M. Geppert and Dr. B. Schmack, Magen-Darm-Zentrum Bayreuth, and Dr. H. Bordel, Dr. R. Müller and Dr. B. Wigginghaus, Gastroenterologische Fachpraxis, Osnabrück) collaborated and participants were recruited between December 2011 and May 2012.

During the study period, we invited adult men and women who were scheduled for elective upper gastrointestinal endoscopy to participate in the

*histo*GERD trial. The participants underwent gastroscopy for various reasons. Criterion for exclusion was previous abdominal surgery leading to an abnormal anatomy in the upper gastrointestinal tract, particularly at the GEJ.
Before the procedure, each participant was asked to fill out a questionnaire about the presence and intensity of reflux symptoms for the last month. For detailed information see the patients´ questionnaire in the appendix section.

Ethics Board Approval

The investigation was carried out in accordance with the Declaration of Helsinki. Written informed consent was given by each participant before the procedure. The study was approved by the Institutional Review Boards of the Medical University of Graz (EK 24-052 ex 11/12) and the University of Erlangen (EK 4571 ex 11/12), respectively, and was registered at ClinicalTrials.gov (NCT01576289).

Endoscopy

We used a standardized assessment form to record basic demographic data (patient age and gender), patients´ medication use (with focus on PPI or other acid suppressant drugs), history of drug intake as well as medical indication for endoscopy. The endoscopists classified the indication for endoscopy as follows: (i) *patients evaluated for diseases of the esophagus* reporting heartburn, acid regurgitation, or both at least once a week (according to Lagergren's criteria for GERD [19]) and/or dysphagia, (ii) *patients evaluated for diseases of the stomach* reporting epigastric discomfort and/or pain, and (iii) *patients evaluated for diseases of the small bowel* reporting diarrhea (with or without weight loss), with multiple answers possible. For detailed information see the assessment form for clinical data in the appendix section.

During the procedure, the esophagus, GEJ, and stomach of all participants were examined according to a standardized protocol devised for the study. The GEJ was defined as the most proximal limit of the gastric mucosal folds, i.e. the point where the tubular esophagus meets the gastric folds [6, 7]. The endoscopists were asked to grade the severity of esophagitis according to the modified Los Angeles classification (Figure 2) which focuses on the extent of mucosal breaks,

but also refers to minimal changes [14, 15]. For detailed information see the assessment form for endoscopic data in the appendix section.

Los Angeles classification system with Japanese modifications[5]

Grade	Description
N	Normal mucosa
M	Minimal changes to the mucosa, such as erythema and/or whitish turbidity
A	Nonconfluent mucosal breaks <5 mm in length
B	Nonconfluent mucosal breaks >5 mm in length
C	Confluent mucosal breaks <75% circumferential
D	Confluent mucosal breaks >75% circumferential

Figure 2: Modified Los Angeles classification by Hongo et al. [15] and Hoshihara et al. [45].

Biopsies were systematically taken from the GEJ according to a standardized protocol: At least two specimens each were obtained from the most proximal extent of the gastric folds (greater and lesser curvature) and from the distal esophagus with the aim of obtaining tissue samples from both above and below the SCJ. Specifically, squamous epithelium was sampled within 1 cm above the SCJ. However, when columnar metaplasia was observed in the distal esophagus the biopsies were obtained upwards until squamous epithelium was reached. Thus, a minimum of four biopsies was obtained from each patient, each biopsy fragment of tissue measuring approximately 2 mm in greatest diameter.

All endoscopists were very experienced in the field: most of them had worked in endoscopy units for at least one decade performing more than 500 gastroscopies per year. Before the investigation, all endoscopists were trained in order to familiarize them with the biopsy protocol and, particularly, the assessment form. For endoscopy, four institutions used the OLYMPUS EVIS EXERA II series (Olympus Europe Holding GmbH, Hamburg, Germany) with video gastroscopes GIF-H180 and Q180, respectively. One institution (Osnabrück) used the FUJI EPX-4450HD Electronic Video Endoscopy System with EG-590WR video gastroscopes (Fujifilm Corporation, Tokyo, Japan).

Histopathology

Biopsy specimens were fixed in 4% buffered formalin, embedded in paraffin, cut at 4-5 levels as 2 µm thick sections and stained with hematoxylin and eosin (H&E) for histologic examination. All histological sections were assessed by two experienced gastrointestinal pathologists (Dr. Michael Vieth and Univ. Doz. Dr. Cord Langner) who were blinded to clinical data and endoscopic findings.

Esophageal biopsies obtained proximal to the SCJ were examined for the presence or absence of microscopic esophageal lesions related to GERD according to recently published consensus guidelines (Esohisto project) [13, 40]. These guidelines provide a systematic basis to score the extent of various histopathological features of the squamous epithelium, such as basal cell layer hyperplasia, papillary elongation, dilation of intercellular spaces, and the presence of intraepithelial inflammatory cells (intraepithelial eosinophils, intraepithelial neutrophils and intraepithelial mononuclear cells). A brief summary for the assessment and scoring of these microscopic changes of the squamous epithelium connected to GERD are presented in Table 1 and a detailed description is given below.

Basal cell layer hyperplasia

To assess the thickness of the basal cell layer it is required to find an eligible area of the squamous epithelium with an intact surface [13, 40]. Generally, the basal cell layer thickness is measured and compared to the total epithelial thickness [13, 21, 40]. In normal esophageal mucosa the basal cell layer comprises of approximately 2-6 cell layers (<15% of total epithelial thickness; Figure 3) of clustered cells with round nuclei appearing slightly basophil [40, 46]. Another landmark is the upper limit of the basal cell layer which is characterized as the level above which >50% of the nuclei are separated by a distance greater than the nuclear diameter [13, 21]. Figure 4 exemplifies the evaluation of basal cell layer hyperplasia.

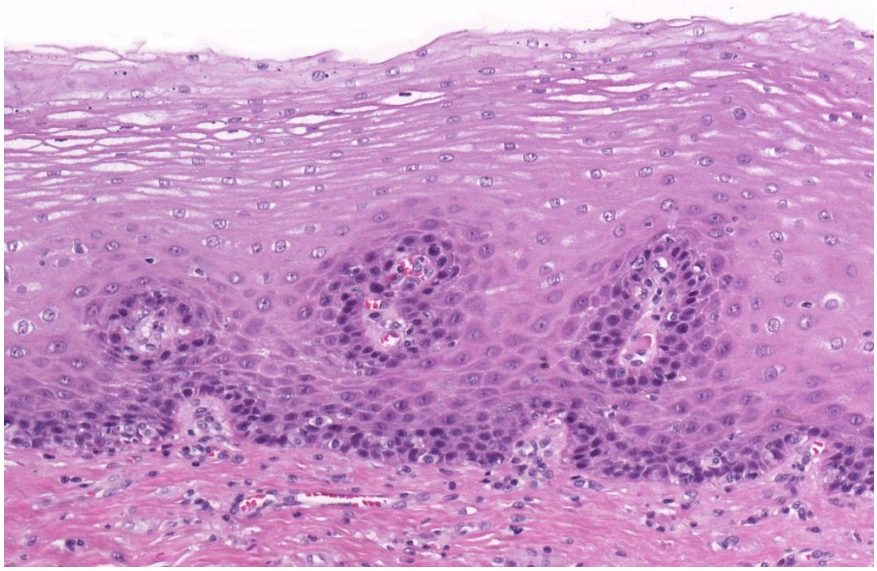


Figure 3: Normal squamous epithelium of the esophagus (original x 100).

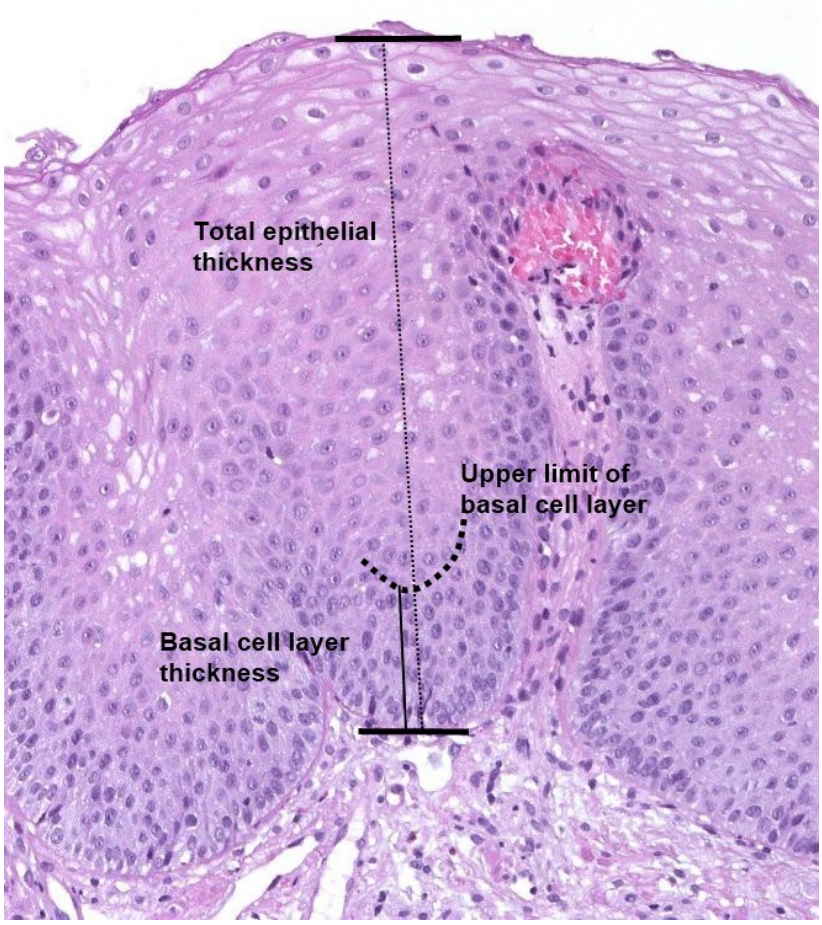


Figure 4: Evaluation method of basal cell hyperplasia (original x 100).

Criterion	Definition and method of assessment	Severity score
Basal cell layer hyperplasia	Measure basal cell layer thickness in µm and express as a proportion (%) of total epithelial thickness (x10)	**0** (<15%), **1** (15–30%), **2** (>30%)
Papillary elongation	Measure papillary length in µm and express as a proportion (%) of total epithelial thickness (x10)	**0** (<50%), **1** (50–75%), **2** (>75%)
Dilation of intercellular spaces	Identify as irregular round dilations or diffuse widening of intercellular space (x40)	**0** (absent), **1**(small;<1 lymphocyte), **2** (large;≥1 lymphocyte)
Intraepithelial eosinophils	Count in the most affected high-power field (x40)	**0** (absent), **1** (1–2 cells), **2** (>2 cells)
Intraepithelial neutrophils	Count in the most affected high-power field (x40)	**0** (absent), **1** (1–2 cells), **2** (>2 cells)
Intraepithelial mononuclear cells	Count in the most affected high-power field (x40)	**0** (0–9 cells), **1** (10–30 cells), **2** (>30 cells)

Table 1: Histologic assessment criteria for microscopic changes in GERD according to the Esohisto project [13, 40].

Papillary elongation

The length of the papilla should be evaluated in an area where the base of the papilla can be explicitly identified surrounded by the most compacted basal cell layer [13, 40]. In reference to the total epithelial thickness, the distance between the upper limit of the central vessel wall and the base of the papilla is measured in µm [13, 21, 40, 46]. The most extended papilla approaching the epithelial surface should be evaluated. In healthy esophageal squamous epithelium (compare Figure 3) is the papillary length <50% of the total epithelial thickness [40, 41]. Figure 5 exemplifies the evaluation of basal cell layer hyperplasia.

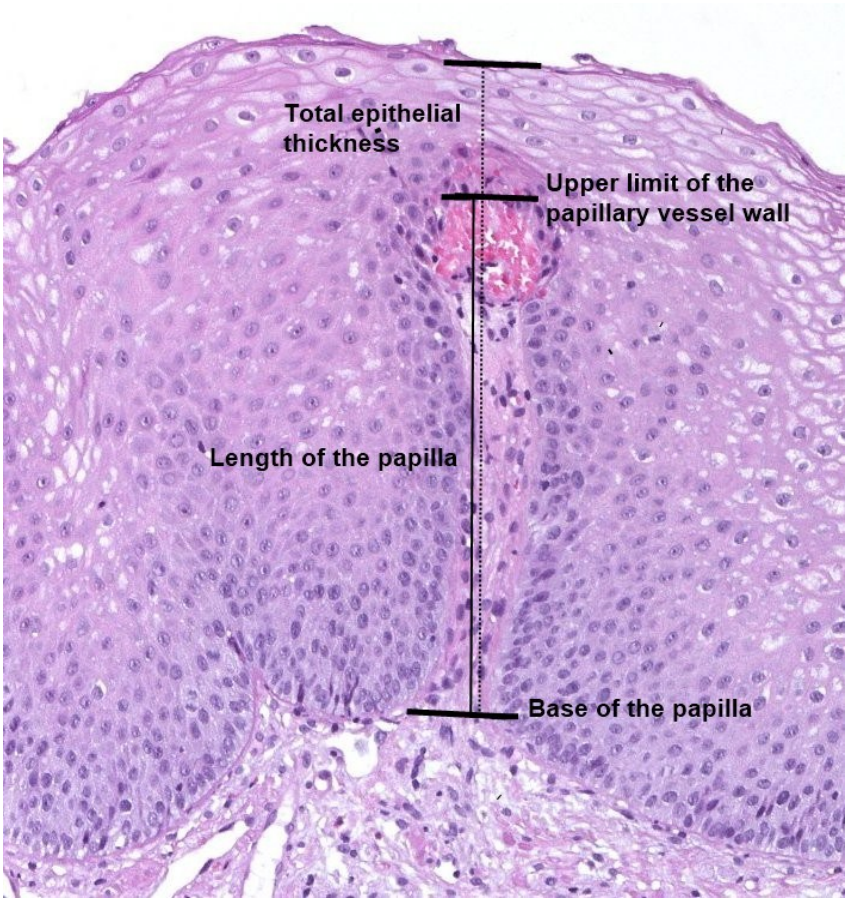


Figure 5: Evaluation method of papillary elongation (original x 100).

Dilation of intercellular spaces

Detached interepithelial cell junctions of the squamous epithelium result in irregular round or diffuse spaces between epithelial cells upon light microscopy. Intracellular vacuoles and artificial cell partition can raise difficulties in assessing dilated intercellular spaces correctly [13, 40]. The severity is evaluated in proportion to the diameter of a lymphocyte [13, 40, 47]. Figure 6 shows an example of dilated intercellular spaces.

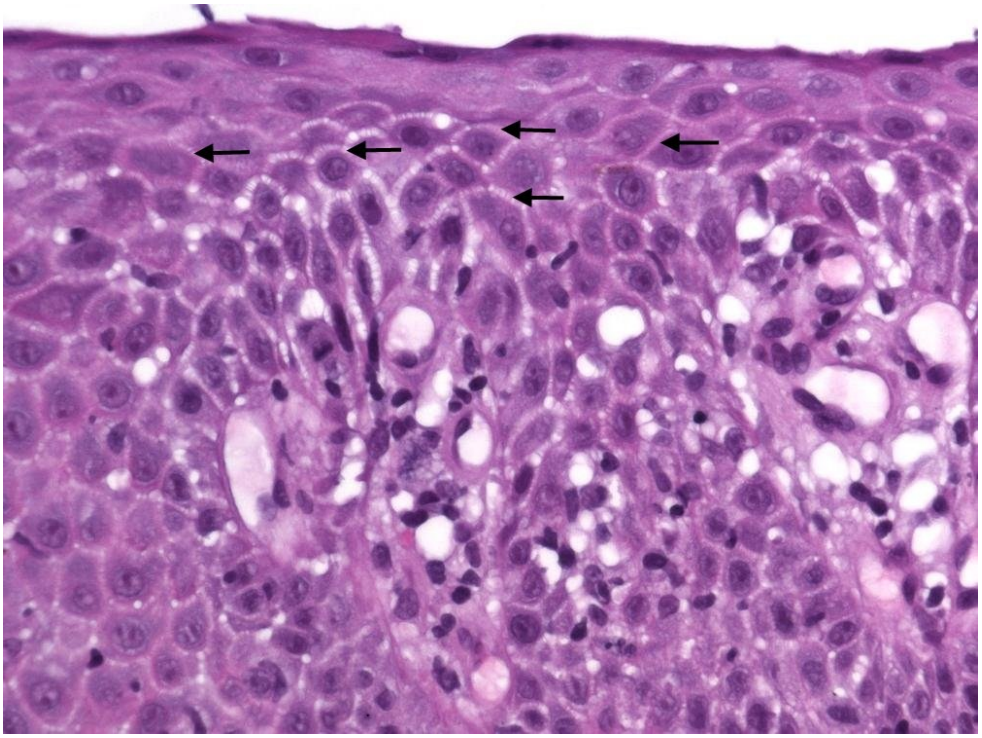


Figure 6: Dilated intercellular spaces (arrows; original x 200).

Infiltration of intraepithelial inflammatory cells

Inflammatory cells (polymorph or mononuclear) inside the squamous epithelium are counted in one high-power field [34, 40, 41]. Mononuclear cells within the connective tissue of the papillae should not be counted [32, 34, 40, 41]. Figure 7 shows an example of infiltrations eosinophils and mononuclear cells.

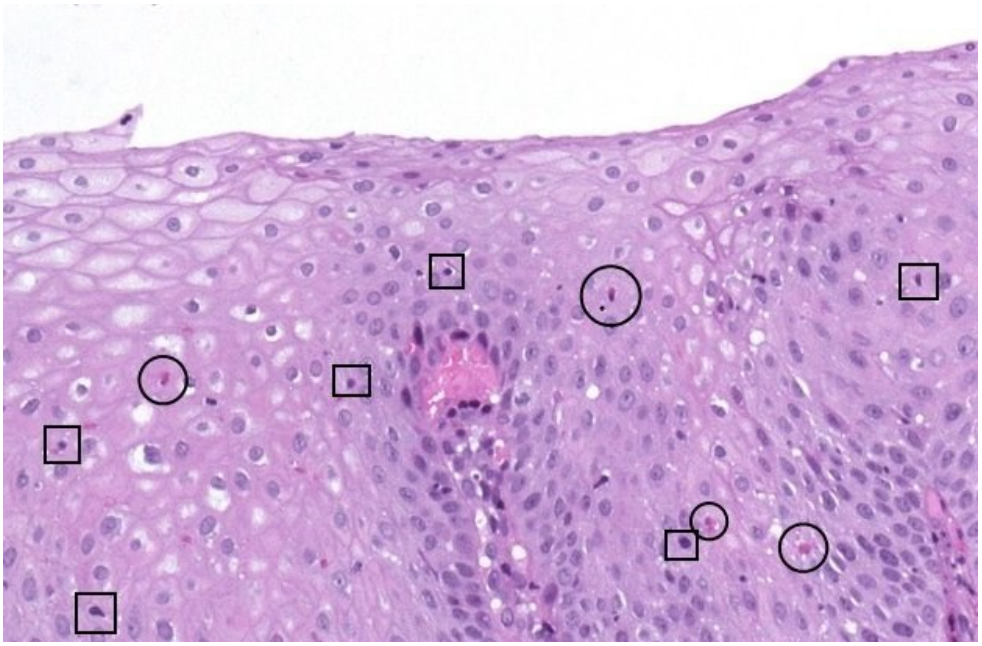


Figure 7: Intraepithelial eosinophils (circle) and intraepithelial mononuclear cells (square; original x 100).

Combined severity score

In addition to assessing the severity of individual lesions, a combined severity score was also obtained for each patient by summing up lesion scores and dividing by the number of lesion types assessed. As suggested in the Esohisto project, the calculation was restricted to basal cell layer hyperplasia, papillary elongation, dilation of intercellular spaces, and the presence of intraepithelial eosinophils as these are the most informative elementary lesions. The score ranges from 0-2. Scores 0-0.25 were regarded as normal, scores 0.5-0.75 qualified for diagnosis of “mild” esophagitis, and scores ≥1 for diagnosis of “severe” esophagitis, respectively [40, 41]. Illustrative images and assessment of the combined severity score are shown in our case study section (Figure 23).

Esophageal biopsies obtained from below the SCJ were examined for different types of columnar epithelium and classified based upon the type of glands in the underlying part of the mucosa, with multiple answers possible. Intestinal metaplasia was diagnosed when goblet cells were present in either the surface or the crypt epithelium.

The columnar epithelium of the esophagus can be classified in three epithelial types originally described by Paull et al. [48] in 1976 with further modifications made by Chandrasoma et al. [35]:

1) Pure gastric oxyntic mucosa (OM) is formed of glands composed entirely of parietal and chief cells without mucous cells (Figure 8) [49].
2) Pure CM contains glands composed of mucous cells only without parietal cells (Figure 9) [49].
3) OCM contains glands with a mixture of mucous cells and parietal cells (Figure10) [49].

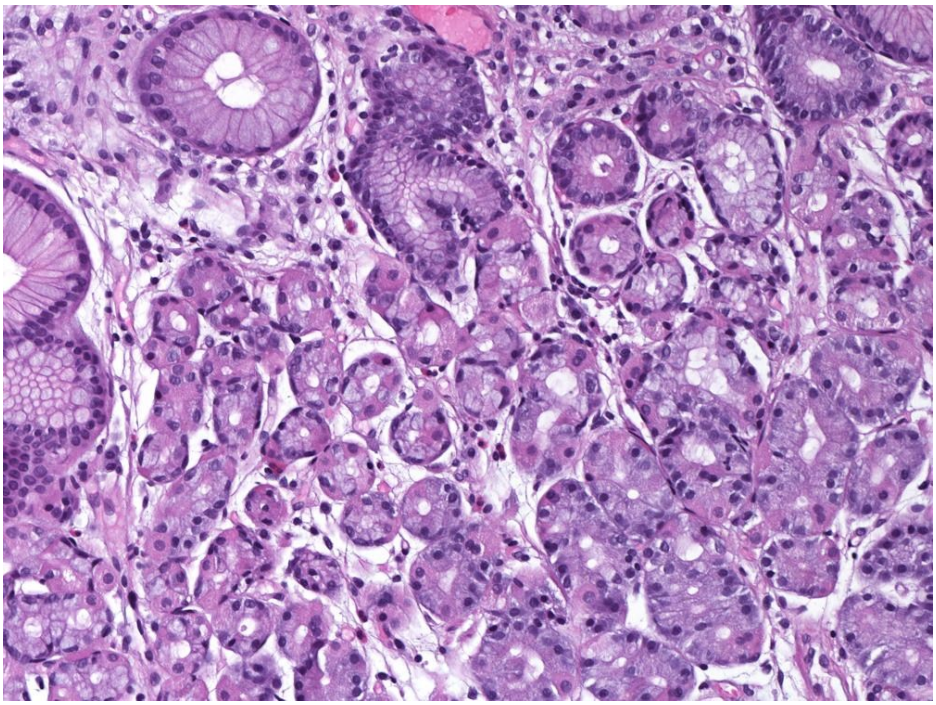


Figure 8: Oxyntic mucosa at the GEJ containing glands of chief and parietal cells (original x 100).

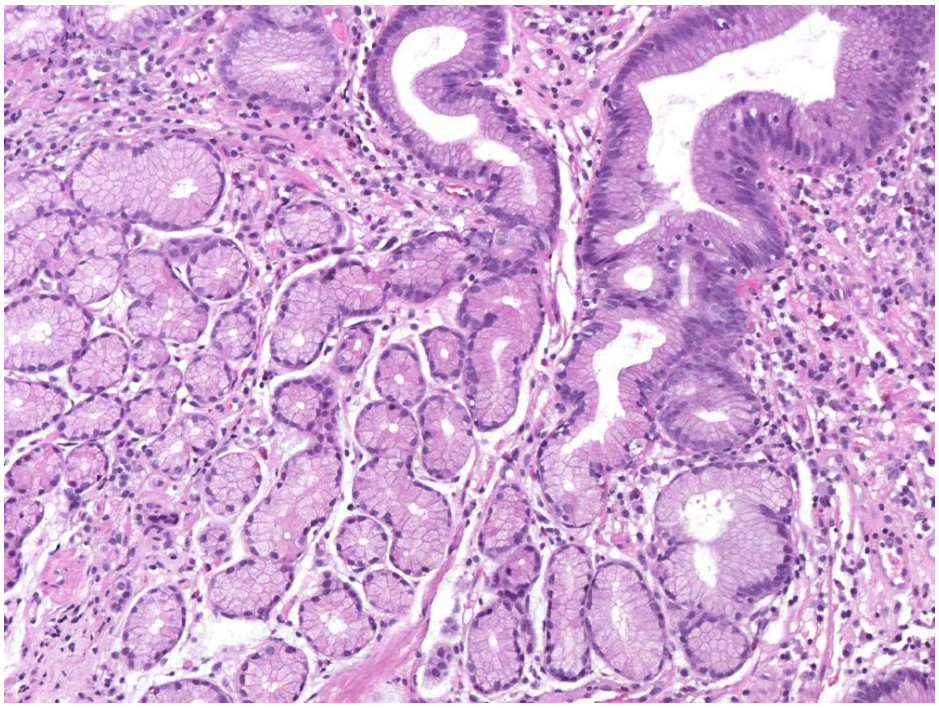


Figure 9: Cardiac mucosa at the GEJ composed of mucous cells only (original x 100).

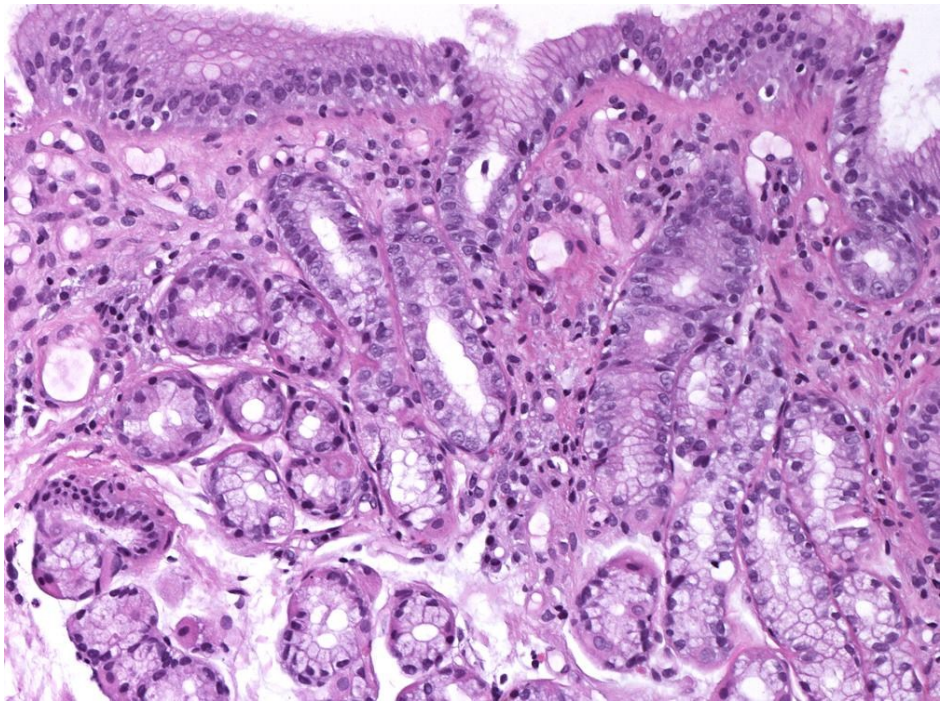


Figure 10: Oxyntocardiac mucosa at the GEJ with glands containing a mixture of parietal and mucous cells (original x 100).

Statistical Analysis

All data were included within a prospective joint database. The quality of this database was tested by random sample taking in 5% of the recruited patients which revealed misreporting in 0.14%. Categorical variables are presented as absolute and relative frequencies, numerical variables as medians and ranges, as well as means. Differences in categorical variables were examined using the chi-square test or Fisher's exact test, as appropriate. Differences in continuous variables between groups were analyzed using the Mann-Whitney U-Test. All statistical calculations were performed using NCSS: Hintze, J. (2007). NCSS, LLC. Kaysville, Utah, U.S.A. (www.ncss.com). Two-sided p-values <0.05 were considered statistically significant [42, 43, 49, 50].

Results

Patient Characteristics

In all, 1,130 individuals participated in the study. Fifty-nine individuals were excluded from the study due to insufficient biopsy sampling: Six individuals were not endoscoped according to protocol with less than four biopsy samples from the GEJ. Likewise 35 individuals had no squamous epithelium and 18 individuals had no columnar epithelium in their biopsy samples. Consequently, the rate of participation was approximately 94.8% leading to a final study cohort of 1,071 participants with sufficient biopsy material from the GEJ (Figure 11).

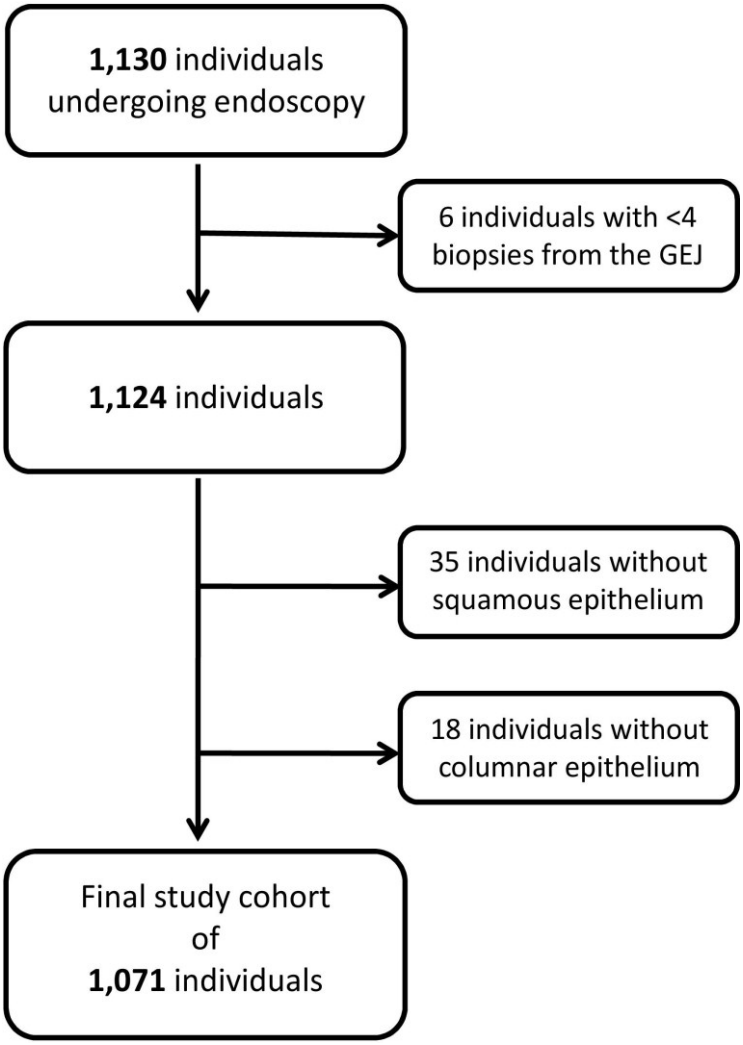


Figure 11: Final study cohort for the evaluation of microscopic esophagitis (*histo*GERD trial) [50].

In our study cohort was a slight female predominance. There were 576 (53.8%) females and 495 (46.2%) males (ratio = 1.16:1). Median age was 53 years (mean 52.1) with a range from 15 to 93 years. You can see the stratification of males and females in different age groups in Figure 12.

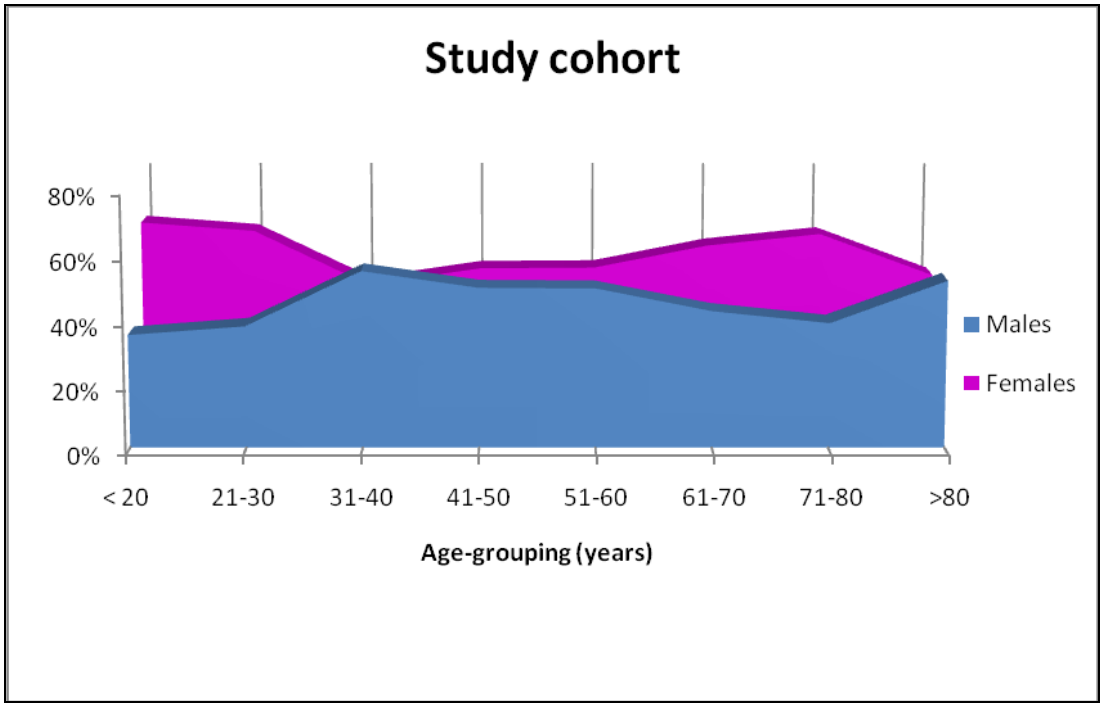


Figure 12: Gender stratification in different age groups.

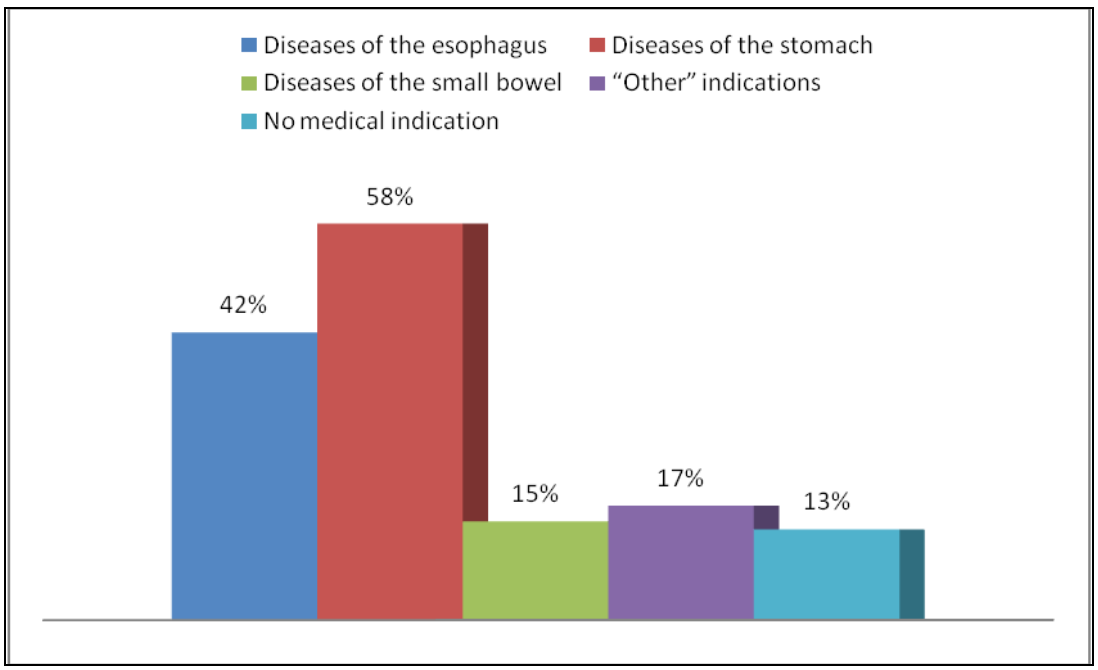


Figure 13: Medical indication for gastroscopy.

When the medical indications of the participants were grouped by the endoscopists, 452 (42.2%) individuals were evaluated for diseases of the esophagus, 623 (58.2%) for diseases of the stomach, and 155 (14.5%) for diseases of the small bowel, respectively. In 180 (16.8%) individuals the endoscopists referred to “other” indications, such as preoperative endoscopy (e.g. prior to cholecystectomy, bariatric surgery, or organ transplantation), evaluation of individuals with inflammatory bowel disease for upper gastrointestinal tract involvement, and evaluation of individuals with anemia, weight loss, and metastatic disease with unknown primary. In 142 (13.3%) individuals no medical indication for gastroscopy was provided (Figure 13).

According to the patients´ questionnaire, 307 (28.7%) individuals reported on heartburn at least once a week and 413(38.6%) individuals on acid regurgitation. Using Lagergren’s criteria [19], with heartburn or acid regurgitation, or both at least once a week, we obtained a subgroup of 520 (48.6%) individuals with symptoms of GERD (Figure 14).

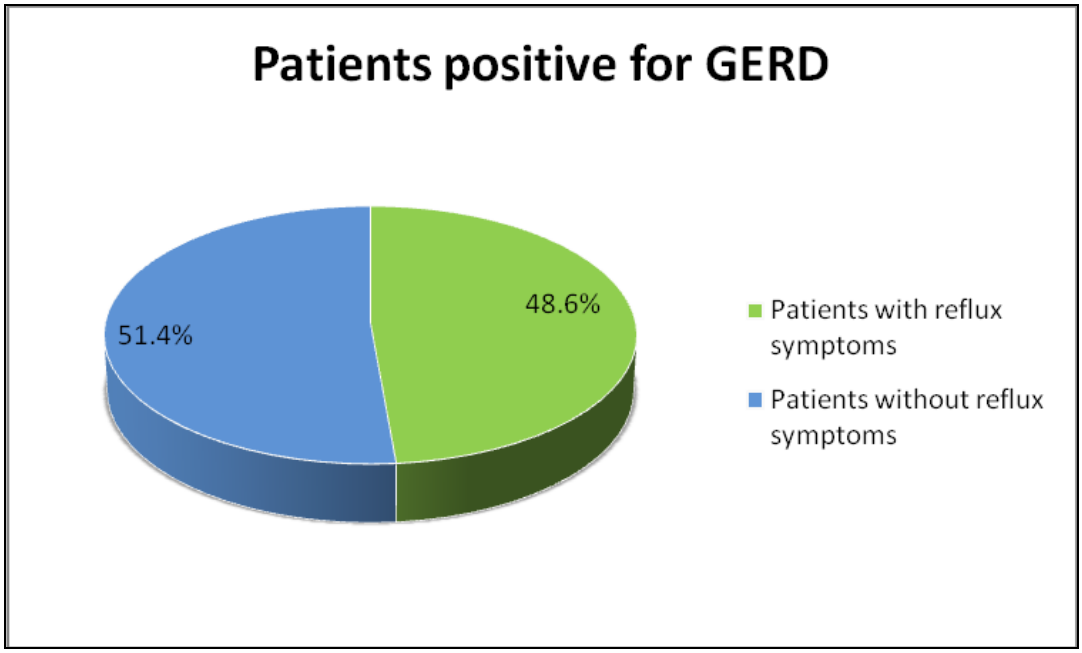


Figure 14: Patients with GERD symptoms in the study cohort.

At time of investigation, 503 (47%) individuals were on therapy with PPIs, with 330 (65.6%) individuals receiving PPIs on a regular basis (Figure 15). For 157 out of 330 individuals with regular PPI therapy, data regarding the duration of PPI intake were available. Mean and median values for the duration of PPI intake were 31.4 and 12 months, respectively.

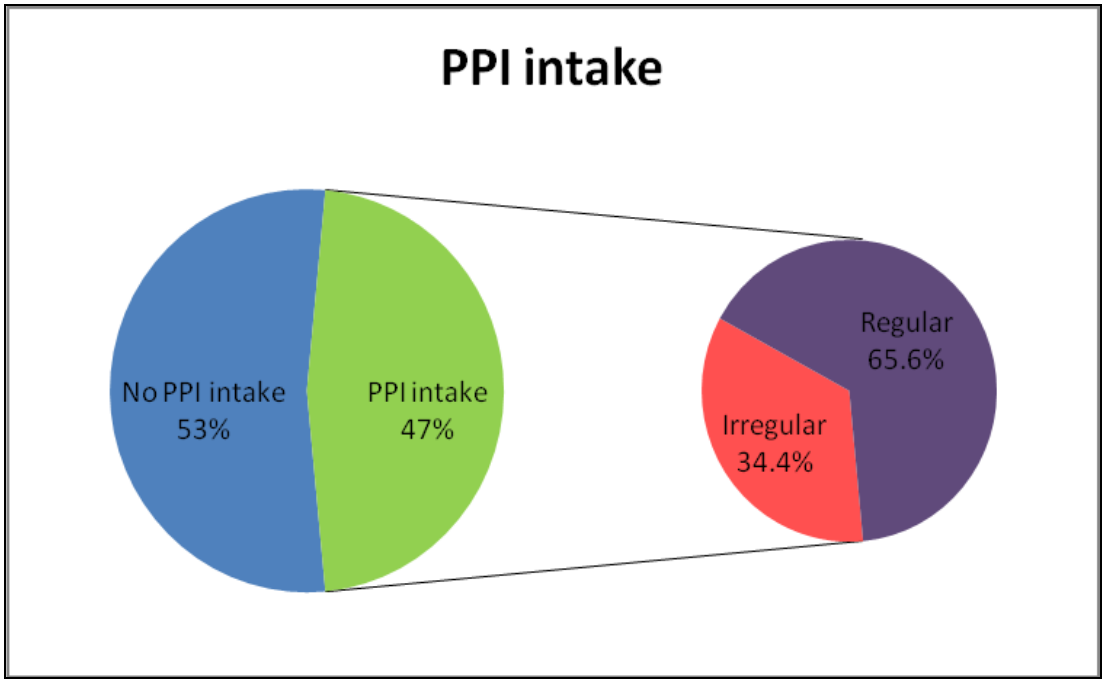


Figure 15: Distribution of PPI intake in the study cohort.

Histologic Diagnosis of Esophagitis

Altogether, histological features indicating GERD were present in a considerable number of individuals. Thus, basal cell hyperplasia, papillary elongation, and dilation of intercellular spaces were noted in 744 (69.5%), 715 (66.8%), and 588 (54.9%) individuals, respectively. The infiltration of inflammatory cells, such as intraepithelial eosinophils, intraepithelial neutrophils and intraepithelial mononuclear cells was observed in 92 (8.6%), 42 (3.9%), and 510 (47.6%) individuals, respectively (Figure 16).

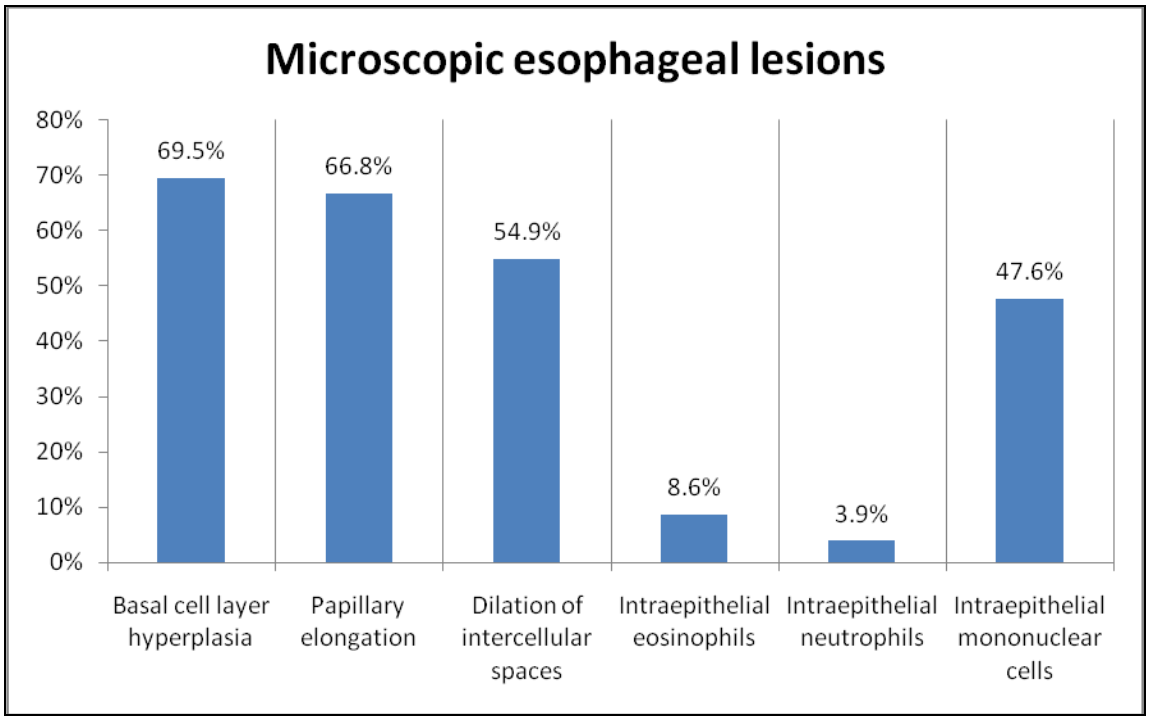


Figure 16: Prevalence of microscopic esophageal lesions.

Applying the combined severity score for the diagnosis of microscopic esophageal lesions, the squamous mucosa was normal upon histology (or showed only insignificant findings) in 352 (32.9%) individuals, was diagnosed with mild esophagitis in 423 (39.5%) individuals or was diagnosed with severe esophagitis in 296 (27.6%) individuals (Figure 17). Examples of microscopic esophagitis according to the combined severity score in patients with GERD are shown in the case studies Figure 23.

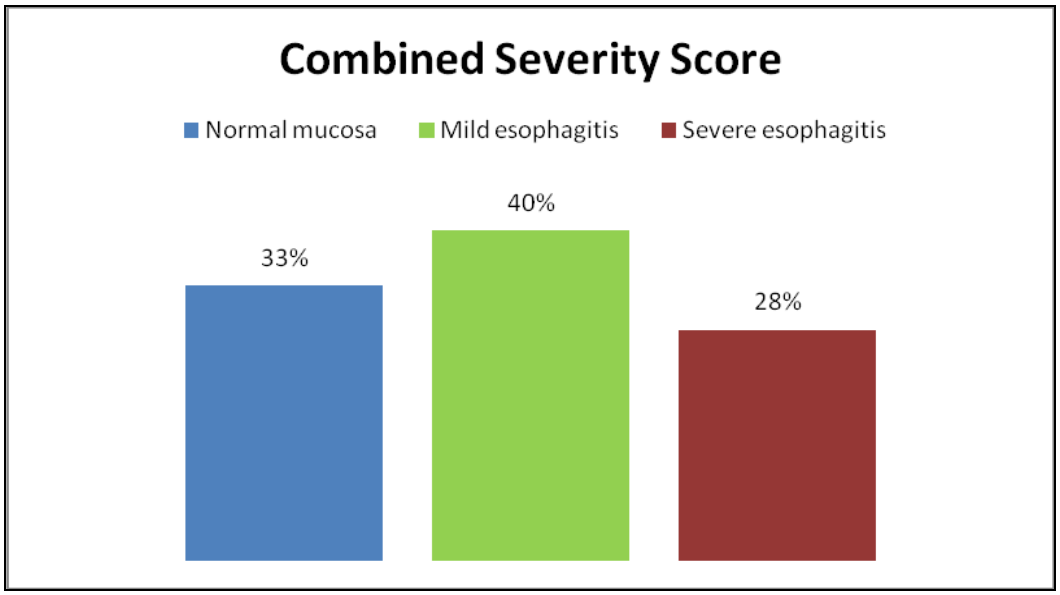


Figure 17: Prevalence of esophagitis in the study cohort according to the combined severity score.

Median age of individuals with normal histology, mild esophagitis, and severe esophagitis was 55 (mean 53.9; range 15-91), 53 (mean 52.2; range 15-93), and 50 (mean 49.7; range 15-85) years, respectively. Prevalence and severity of esophagitis was significantly associated with gender, as 42.3% of individuals with normal histology were males, compared to 44.2% with mild and 53.7% with severe esophagitis (p=0.009; Figure 18).

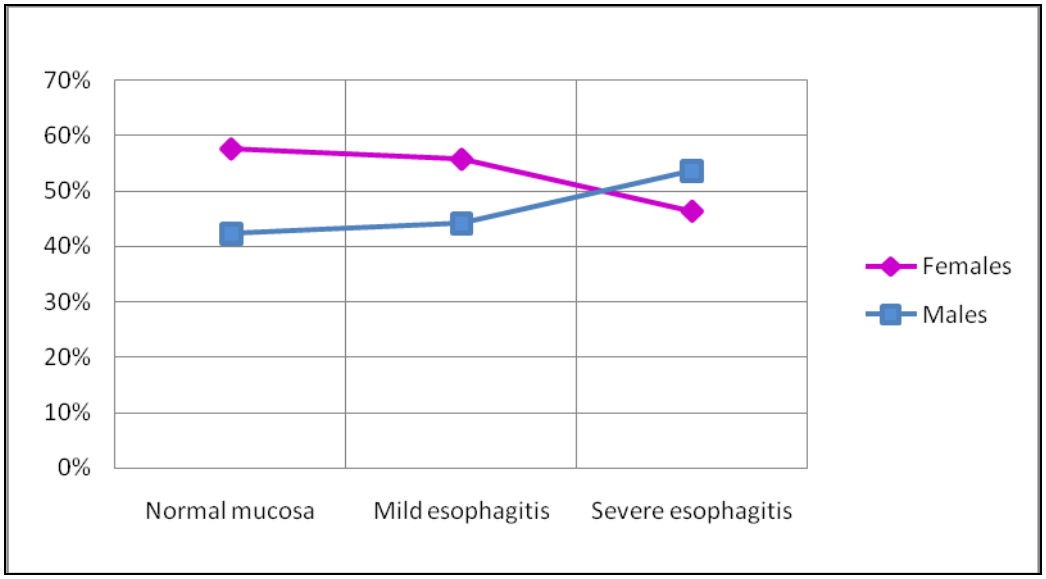


Figure 18: Association with the severity of esophagitis and gender.

The microscopic diagnosis of esophagitis was significantly associated with patients' symptoms. This holds true for the combined severity score (Figure 19) as well as the following elementary lesions: basal cell layer hyperplasia, papillary elongation, and presence of intraepithelial neutrophils. Regarding the most frequently elementary lesions, basal cell layer hyperplasia and papillary elongation, 333 (73.7%) and 324 (71.7%) individuals reported on symptoms of GERD, compared to 119 (26.3%) and 128 (28.3%) individuals without symptoms of GERD, respectively. Further details are presented in Table 2.

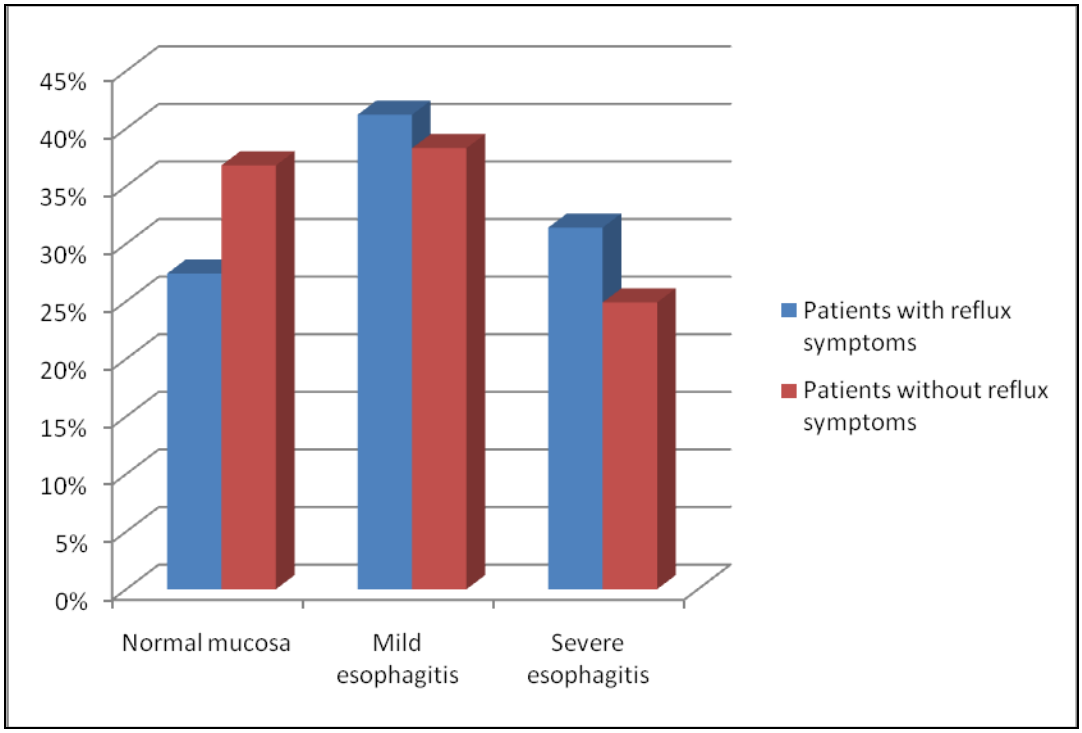


Figure 19: Relation of microscopic esophagitis and patients´ symptoms.

Criterion	Severity score	Symptoms of esophageal disease		P-Value
		Absent (n= 619)	Present (n=452)	
Basal cell layer hyperplasia	0 1 2	208 (33.6%) 281 (45.4%) 130 (21%)	119 (26.3%) 210 (46.5%) 123 (27.2%)	0.012
Papillary elongation	0 1 2	228 (36.8%) 271 (43.8%) 120 (19.4%)	128 (28.3%) 202 (44.7%) 122 (27%)	0.0019
Dilation of intercellular spaces	0 1 2	292 (47.2%) 265 (42.8%) 62 (10%)	191 (42.3%) 211 (46.7%) 50 (11.1%)	0.28
Intraepithelial eosinophils	0 1 2	568 (91.8%) 27 (4.4%) 24 (3.9%)	411 (90.9%) 24 (5.3%) 17 (3.8%)	0.77
Intraepithelial neutrophils	0 1 2	601 (97.1%) 5 (0.8%) 13 (2.1%)	428 (94.7%) 12 (2.7%) 12 (2.7%)	0.047
Intraepithelial mononuclear cells	0 1 2	341 (55.1%) 253 (40.9%) 25 (4%)	220 (48.7%) 210 (46.5%) 22 (4.9%)	0.11
Combined severity score	Normal Mild Severe	228 (36.8%) 237 (38.3%) 154 (24.9%)	124 (27.4%) 186 (41.2%) 142 (31.4%)	0.003

Table 2: Histologic criteria according to Esohisto guidelines and combined severity score related to the presence of reflux symptoms [50].

In addition, prevalence and severity of histologic findings was inversely associated with PPI intake. Thus, patients receiving PPIs, either on demand or on a regular basis showed significantly milder histologic changes (Table 3; Figure 20).

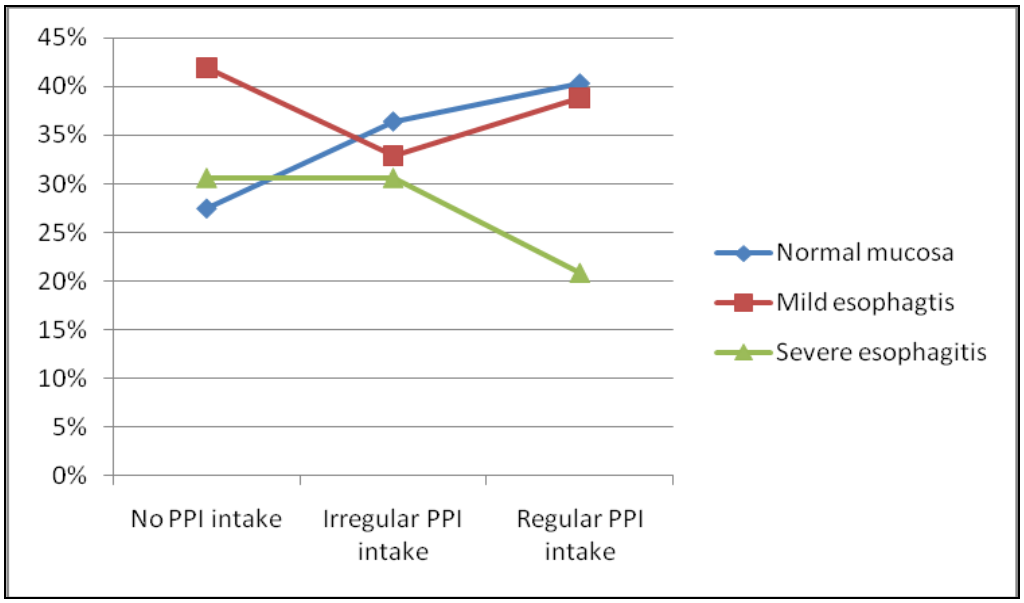


Figure 20: PPI intake and severity of esophagitis.

	Severity score	PPI intake			P-Value
		No PPI (n= 568)	Irregular (n=173)	Regular (n=330)	
Basal cell layer hyperplasia	0 1 2	146 (25.7%) 268 (47.2%) 154 (27.1%)	59 (34.1%) 72 (41.6%) 42 (24.3%)	122 (37%) 151 (45.8%) 57 (17.3%)	0.001
Papillary elongation	0 1 2	155 (27.3%) 267 (47%) 146 (25.7%)	63 (36.4%) 69 (39.9%) 41 (23.7%)	138(41.8%) 137(41.5%) 55 (16.7%)	<0.001
Dilation of intercellular spaces	0 1 2	231 (40.7%) 268 (47.2%) 69 (12.1%)	84 (48.6%) 69 (39.9%) 20 (11.6%)	168(50.9%) 139(42.1%) 23 (7%)	<0.001
Intraepithelial eosinophils	0 1 2	514 (90.5%) 34 (6%) 20 (3.5%)	158 (91.3%) 4 (2.3%) 11 (6.4%)	307(93%) 13(3.9%) 10 (3%)	0.086
Intraepithelial neutrophils	0 1 2	544 (95.8%) 13 (2.3%) 11 (1.9%)	164 (94.8%) 2 (1.2%) 7 (4%)	321 (97.2%) 2(0.6%) 7 (2.1%)	0.15
Intraepithelial mononuclear cells	0 1 2	270 (47.5%) 266 (46.8%) 32 (5.6%)	95 (54.9%) 71 (41%) 7 (4%)	196(59.4%) 126(38.2%) 8(2.4%)	0.005
Combined severity score	Normal Mild Severe	156 (27.5%) 238 (41.9%) 174 (30.6%)	63 (36.4%) 57 (32.9%) 53 (30.6%)	133 (40.3%) 128 (38.8%) 69 (20.9%)	<0.001

Table 3: Histologic criteria according to Esohisto guidelines and combined severity score related to intake of PPIs [50].

When the GEJ was examined according to the type of glands under the columnar epithelium, OM was observed in 522 (48.7%) individuals, OCM in 504 (47.1%) individuals, and CM in 713 (66.6%) individuals, with multiple answers possible.

Referring to the microscopic diagnosis of esophagitis we found an association between the presence of CM and all esophageal lesions according to the Esohisto project, although in varying extent [49]. In the subgroup with CM, basal cell hyperplasia was observed in 561 (78.7%), papillary elongation in 549 (77%) and dilation of intercellular spaces in 462 (64.8%) individuals, respectively, compared to 183 (51.2%), 166 (46.4%) and 126 (35.2%) individuals in the subgroup without CM, respectively ($P < 0.001$) [49]. Details and the severity of histologic changes are presented in Table 4.

Furthermore, presence of CM was significantly associated with the combined severity score ($p<0.001$). In the subgroup with CM 190 (53.1%) individuals showed a normal esophageal mucosa, 96 (26.8%) individuals were diagnosed with mild and 72 (20.1%) with severe esophagitis, while in the subgroup without CM, normal mucosa, mild esophagitis and severe esophagitis was diagnosed in 162 (22.7%), 327 (45.9%) and 224 (31.4%) individuals, respectively.

Criterion		Individuals *without* cardiac mucosa (n=358)		Individuals *with* cardiac mucosa (n=713)		p-value
	Severity	**N**	**%**	**N**	**%**	
Basal cell layer hyperplasia	0	175	48.9	152	21.3	<0.001
	1	128	35.8	363	50.9	
	2	55	15.4	198	27.8	
Papillary elongation	0	192	53.6	164	23.0	<0.001
	1	112	31.3	361	50.6	
	2	54	15.1	188	26.4	
Dilation of intercellular spaces	0	232	64.8	251	35.2	<0.001
	1	83	23.2	393	55.1	
	2	43	12.0	69	9.7	
Intraepithelial mononuclear cells	0	268	74.9	293	41.1	<0.001
	1	70	19.6	393	55.1	
	2	20	5.6	27	3.8	
Intraepithelial eosinophils	0	321	89.7	658	92.3	0.047
	1	16	4.5	35	4.9	
	2	21	5.9	20	2.8	
Intraepithelial neutrophils	0	340	95	689	96.6	0.038
	1	4	0.1	13	1.8	
	2	14	3.9	11	1.5	

Table 4: Cardiac mucosa related to histological findings indicative of GERD [49].

Endoscopic Diagnosis of Esophagitis

The prevalence and severity of esophagitis was assessed according to the modified Los Angeles classification [14, 15] as follows: 450 (42%) were normal (Category N) upon endoscopic inspection, 303 (28.3%) individuals presented with minimal changes (Category M) and 318 (29.7%) with mucosal breaks of different extent (Categories A-D; Figure 21).

Demonstrative images of the endoscopic and histologic diagnoses of reflux esophagitis are shown in our case studies (Figure 23).

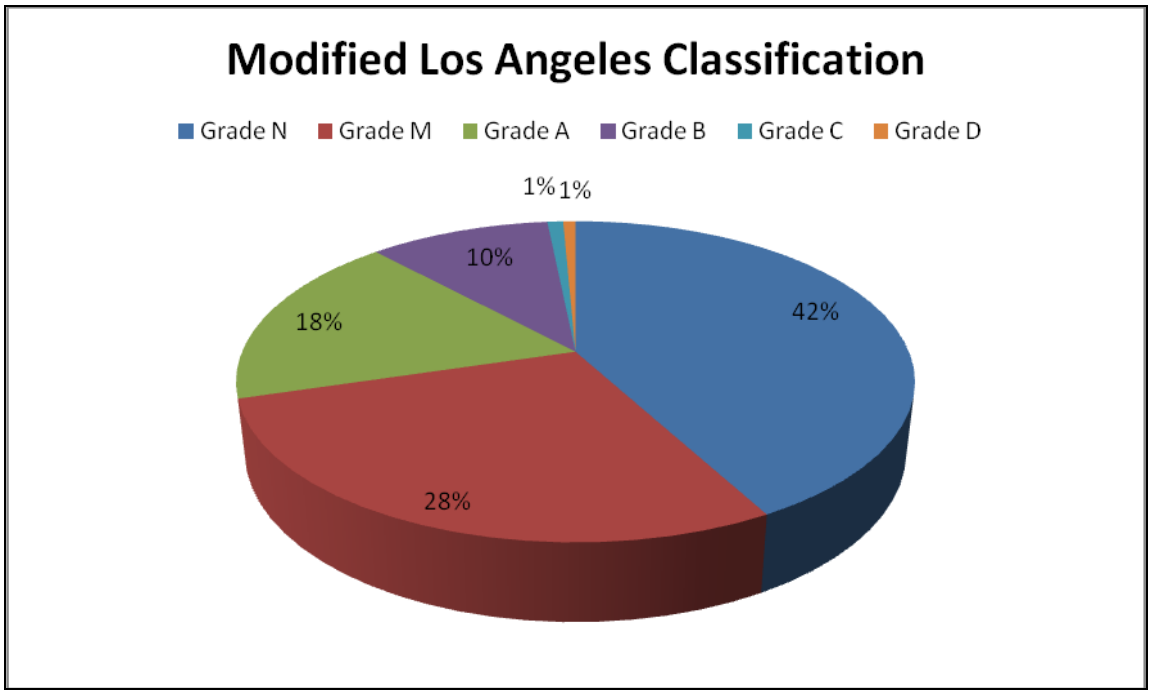


Figure 21: Distribution of the endoscopic diagnosis of esophagitis according to the modified Los Angeles classification.

Relation between Histologic and Endoscopic Diagnosis of Esophagitis

The endoscopic diagnosis of esophagitis was significantly associated with patients' symptoms: 136 (30.2%) individuals with normal mucosa (Category N), 140 (46.2%) individuals with minimal changes (Category M), and 176 (55.3%) individuals with mucosal breaks (Category A-D) reported on heartburn, acid regurgitation, and/or dysphagia ($p<0.001$).

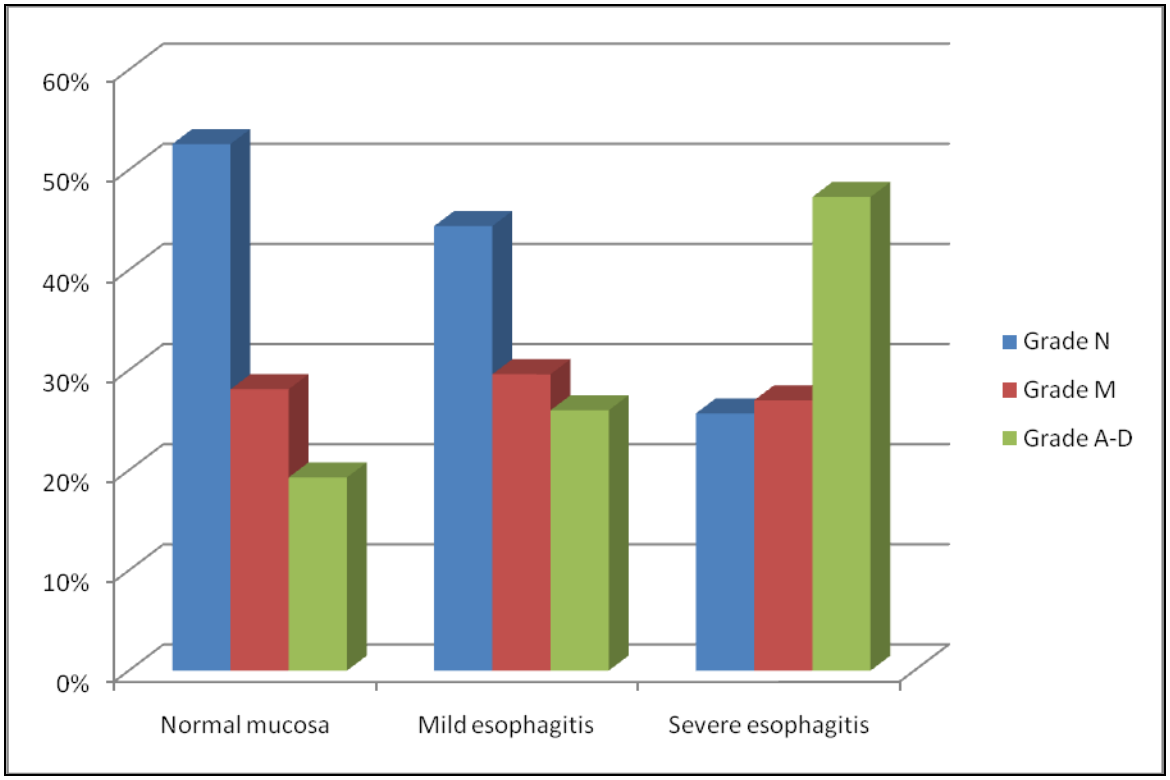


Figure 22: Relation of the combined severity score to the endoscopic diagnosis of esophagitis according to the modified Los Angeles classification.

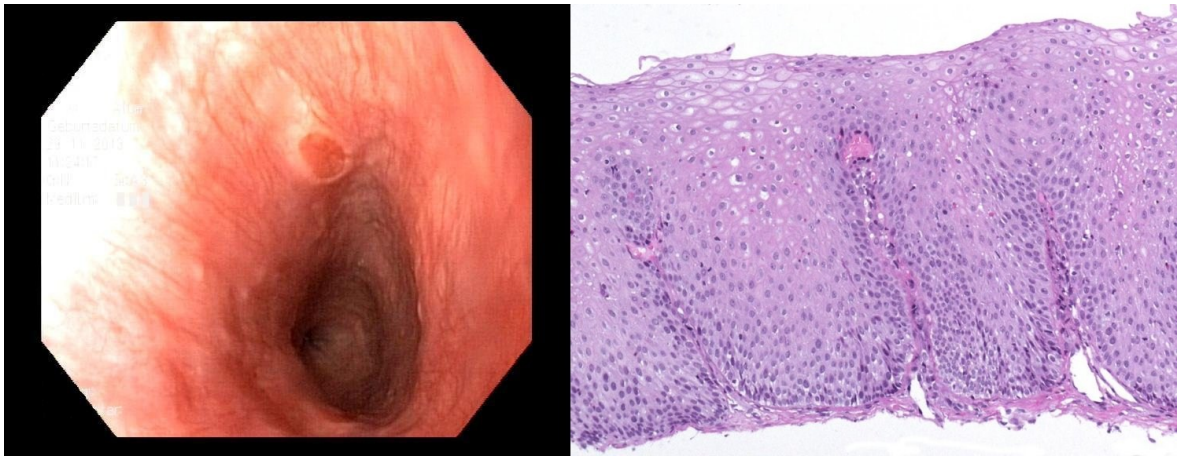
When the histologic diagnosis of both elementary lesions and combined severity score was related to the endoscopic diagnosis of esophagitis, a highly significant association was identified. Regarding the combined severity score, in 185 (52.6%) individuals with normal mucosa (Category N), 99 (28.1%) individuals with minimal changes (Category M), and 68 (19.3%) individuals with mucosal breaks (Category A-D), the squamous mucosa was normal upon histology. Further, 77 (26%) individuals with normal mucosa (Category N), 79 (26.7%) individuals with minimal changes (Category M), and 140 (47.3%) individuals with mucosal breaks (Category A-D), were diagnosed with severe esophagitis upon histology (Figure 22). More details are presented in Table 5.

Table 5: Histologic criteria according to Esohisto guidelines and combined severity score related to the endoscopic diagnosis of esophagitis, graded according to the modified Los Angeles classification [50].

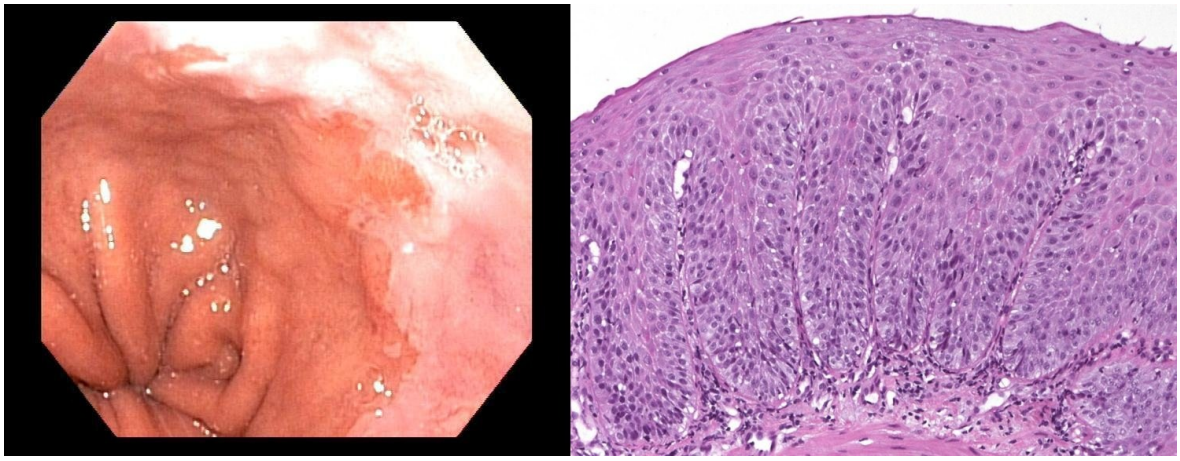
Criterion	Severity Score	Endoscopic Diagnosis of Esophagitis						P-Value
		N (n=450)	M (n=303)	A (n=190)	B (n=110)	C (n=10)	D (n=8)	
Basal cell layer hyperplasia	0 1 2	170 (37.8%) 221 (49.1%) 59 (13.1%)	93 (30.7%) 141 (46.5%) 69 (22.8%)	40 (21%) 86 (45.3%) 64 (33.7%)	17 (15.5%) 38 (34.5%) 55 (50%)	4 (40%) 2 (20%) 4 (40%)	3 (37.5%) 3 (37.5%) 2 (25%)	<0.001
Papillary elongation	0 1 2	186 (41.3%) 203 (45.1%) 61 (13.6%)	100 (33%) 141 (246.5%) 62 (20.5%)	46 (24.2%) 83 (43.7%) 61 (32.1%)	17 (15.5%) 41 (37.3%) 52 (47.3%)	4 (40%) 2 (20%) 4 (40%)	3 (37.5%) 3 (37.5%) 2 (25%)	0.027
Dilation of intercellular spaces	0 1 2	229 (50.9%) 195 (43.3%) 26 (5.8%)	148 (48.8%) 135 (44.6%) 20 (6.6%)	68 (35.8%) 88 (46.3%) 34 (17.9%)	29 (26.4%) 52 (47.3%) 29 (26.4%)	5 (50%) 4 (40%) 1 (10%)	4 (50%) 2 (25%) 2 (25%)	<0.001
Intraepithelial eosinophils	0 1 2	423 (94%) 9 (2%) 18 (4%)	283 (93.4%) 9 (3%) 11 (3.6%)	172 (90.5%) 16 (8.4%) 2 (1.1%)	86 (78.2%) 15 (13.6%) 9 (8.2%)	9 (90%) 1 (10%) 0 (0%)	6 (75%) 1 (12.5%) 1 (12.5%)	<0.001
Intraepithelial neutrophils	0 1 2	444 (98.7%) 0 (0%) 6 (1.3%)	294 (97%) 2 (0.7%) 7 (2.3%)	182 (95.8%) 6 (3.1%) 2 (1.1%)	94 (85.5%) 7 (6.4%) 9 (8.2%)	10 (100%) 0 (0%) 0 (0%)	5 (50%) 2 (25%) 1 (12.5%)	<0.001
Intraepithelial mononuclear cells	0 1 2	251(55.8%) 186 (41.3%) 13 (2.9%)	162 (53.5%) 128 (42.2%) 13 (4.3%)	98 (51.6%) 86 (45.3%) 6 (3.1%)	42 (38.2%) 55 (50%) 13 (11.8%)	4 (40%) 5 (50%) 1 (10%)	4 (50%) 3 (37.5%) 1 (12.5%)	0.003
Combined severity score	Normal Mild Severe	185 (41.1%) 188 (41.8%) 77 (17.1%)	99 (32.7%) 125 (41.3%) 79 (26.1%)	45 (23.7%) 73 (38.4%) 72 (37.9%)	16 (14.5%) 33 (30%) 61 (55.5%)	4 (40%) 2 (20%) 4 (40%)	3 (37.5%) 2 (25%) 3 (37.5%)	<0.001

Case studies

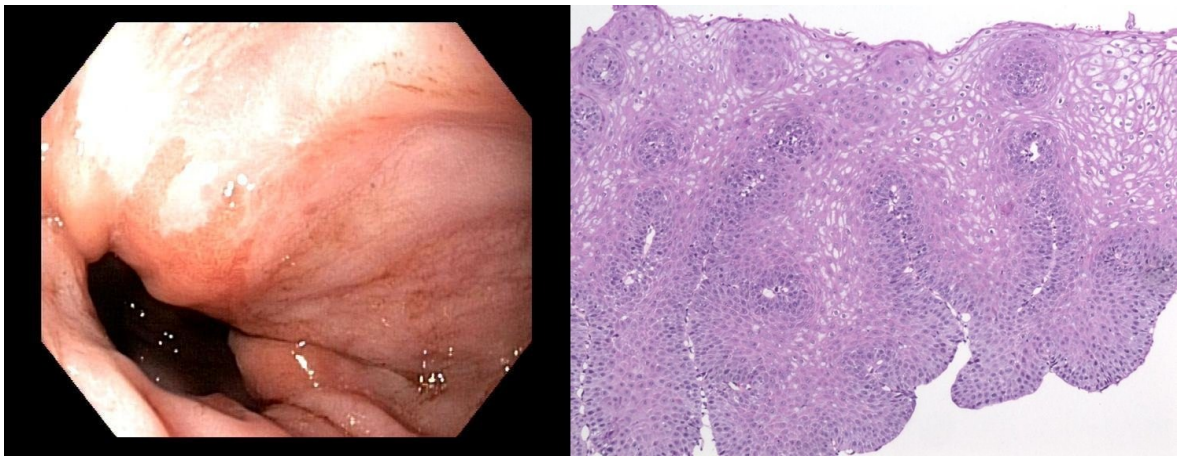
Figure 23: Illustrative images of the endoscopic and histologic diagnoses of GERD. (Endoscopic images courtesy of Dr. Wolfgang Plieschnegger, St. Veit / Glan, Austria).



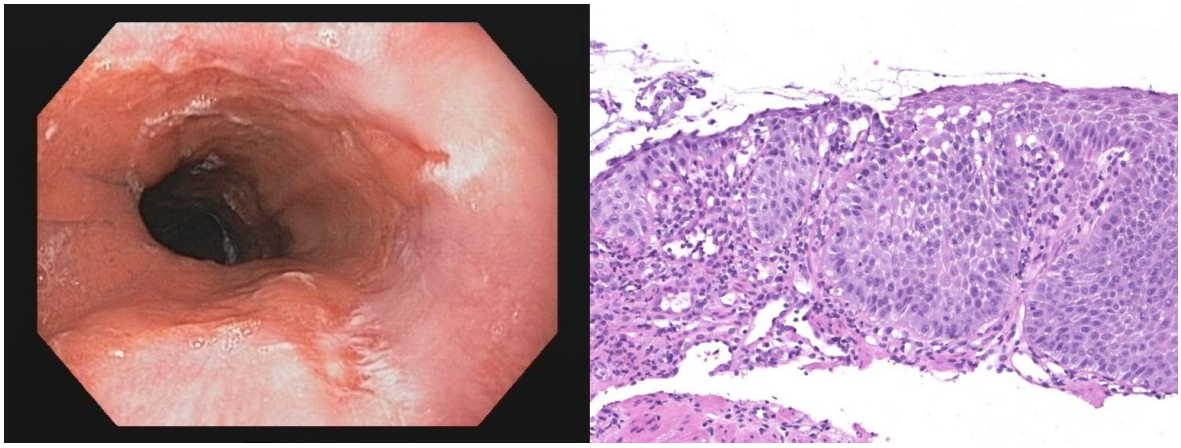
Case 1: A 72-year-old female with minimal changes at the GEJ (Los Angeles grade M) upon endoscopy and mild histologic esophagitis corresponding to a combined severity score of 0.75 (basal cell layer hyperplasia score 1, papillary elongation score 1).



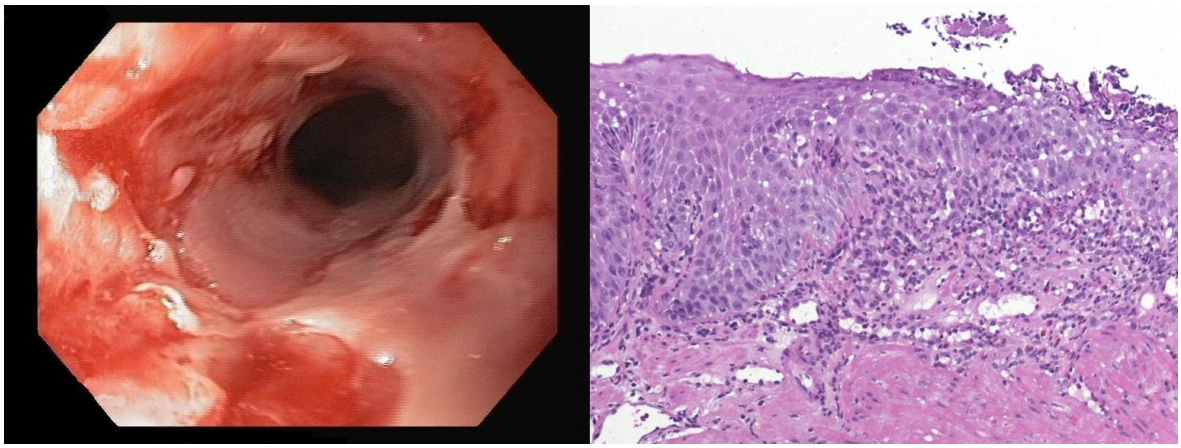
Case 2: A 32-year-old male with Los Angeles grade A upon endoscopy and severe histologic esophagitis corresponding to a combined severity score of 1 (basal cell layer hyperplasia score 2, papillary elongation score 1, dilated intercellular spaces score 1).



Case 3: A 69-year-old male with Los Angeles grade A upon endoscopy and severe histologic esophagitis corresponding to a combined severity score of 1.25 (basal cell layer hyperplasia score 2, papillary elongation score 2, dilated intercellular spaces score 1).



Case 4: A 74-year-old female with Los Angeles grade B upon endoscopy and severe histologic esophagitis corresponding to a combined severity score of 1.5 (basal cell layer hyperplasia score 2, papillary elongation score 2, dilated intercellular spaces score 2).



Case 5: A 62-year-old male with Los Angeles grade D upon endoscopy and severe histologic esophagitis corresponding to a combined severity score of 1.75 (basal cell layer hyperplasia score 2, papillary elongation score 2, dilated intercellular spaces score 2, intraepithelial eosinophils score 1).

Discussion

Various histologic features of the squamous epithelium related to GERD have been identified. These include basal cell layer hyperplasia and papillary elongation [21, 22], presence of intraepithelial neutrophils [29], eosinophils [30, 31] and mononuclear cells [32, 33]. More recently, dilation of intercellular spaces has been identified as another important feature [25-28].

The consensus guidelines presented by the Esohisto project provide an excellent basis for standardized recognition of microscopic esophagitis, including the grading of disease severity. In particular, the guidelines refer to a severity score for the tested individual lesions (ranging from 0 to 2) [13, 40]. In a subsequent paper, aimed at the refinement and reproducibility of histologic criteria, the authors introduced a combined severity score restricted to basal cell layer hyperplasia, papillary elongation, dilation of intercellular spaces, and the presence of intraepithelial eosinophils [40] as these four features are considered to be the most informative elementary lesions [41]. Levels of interobserver agreement were tested for all features. The percentage agreement was 64% for dilation of intercellular spaces, and was in the range 73–74% for basal cell layer hyperplasia, papillary elongation, and intraepithelial mononuclear cells, and 83–97% for intraepithelial eosinophils, intraepithelial neutrophils, active erosions, and healed erosions [40].

In the *histo*GERD trial we systematically evaluated the histologic criteria presented in the Esohisto project, using a standardized biopsy protocol. Specifically, we related the prevalence and severity of microscopic lesions to various clinical and endoscopic features indicative of GERD. The definitions provided in the Esohisto consensus guidelines are simple and proved feasible in all patients. According to our study, the histologic features for diagnosis of esophagitis were significantly associated with patients' symptoms, male gender, endoscopic diagnosis of esophagitis and history of PPI intake.

Previous biopsy studies reported that the finding of various microscopic lesions is unspecific and inconsistent in patients with GERD [38], additionally the sensitivity of histologic features appears to be very low with no significant difference between reflux patients and a control group [37]. Thus, in contrast to previous reports on the marginal role of histology in patients with GERD, our data clearly indicate that, although not routinely recommended in current practice guidelines [12, 51], histology can well serve as a useful diagnostic tool.

In the recent study by Zentilin et al. [39] investigating the diagnostic role of histological alterations in 119 patients with and without symptoms of GERD the sensitivity of histologic features was 96% in patients with erosive esophagitis. In patients with NERD, microscopic lesions indicative of reflux disease were found in 47 of 59 (80%) cases with abnormal pH monitoring, and in 7 of 12 (58%) cases with negative pH monitoring. Comparable results were obtained in our study: Among the 450 patients with no endoscopic signs of esophagitis (Los Angeles Category N), 41.8% were identified with mild, and 17.1% with severe (microscopic) esophagitis, respectively, indicating higher sensitivity of histologic diagnosis. As in our investigation, proliferative changes of the squamous epithelium, in particular basal cell layer hyperplasia and dilation of intercellular spaces were the most common microscopic lesions in the study by Zentilin et al. [39].

Infiltration of the squamous epithelium by eosinophils was more frequently observed than by neutrophils. However, the diagnostic consequence of intraepithelial eosinophils was minor because they did not have an impact on the specificity of histology in the control group. Furthermore, the histological differentiation of GERD patients from the control group was only influenced by the presence of intraepithelial eosinophils in two cases. The authors concluded that the diagnostic role of histology in patients with GERD should be reconsidered, especially in patients with NERD, where histology can additionally provide feasible and objective data in 76% [39].

In our investigation, infiltration of the squamous epithelium by mononuclear cells was found to be more frequent than infiltration by eosinophils or neutrophils. This observation is well in line with the ProGERD study [24], identifying the diagnostic value of histology in a selected study cohort of patients with symptoms of GERD. Comparable to that study, the infiltration by eosinophils and neutrophils

markedly increased with increasing severity of endoscopic diagnosis of esophagitis in our study. In the ProGERD study, the most promising microscopic features, for the diagnosis of GERD and NERD were basal cell layer hyperplasia and papillary elongation. In contrast to our study, the increase in mononuclear cells was rather modest (Los Angeles N-M 45.2% *vs.* Los Angeles A-D 53.5%), suggesting low sensitivity of this marker for the diagnosis of GERD.

In a subsequent publication of the ProGERD study [52] proliferative changes of the squamous epithelium, in particular basal cell layer hyperplasia and papillary elongation were associated with severity of disease. This study was designed to assess the influence of PPI, specifically esomeprazol, on histologic criteria in patients with endoscopically diagnosed GERD. After treatment with esomaprazol (20 mg for patients with NERD and 40 mg for patients with erosive reflux disease) for 4 weeks both thickness of basal cell layers and length of papillae were significantly reduced.

Other data in the literature confirm this observation [53]. In patients with erosive reflux disease, a significantly regression of ulcers and erosions (21% and 31% before treatment vs. 0% and 3% after PPI treatment, respectively) was noted after 12 months of PPI therapy. Correspondingly, basal cell hyperplasia and papillary elongation of the squamous epithelium were observed in 51% before treatment with PPI and were reduced to 3% and 2%, respectively, after PPI treatment.

Additionally, Calabrese et al. [54] reported a complete restitution of dilated intercellular spaces, accompanied by improvement of clinical symptoms, in 92.1% and 97.4% of patients after 3 and 6 months of PPI therapy, respectively.

In the LOTUS trial, a long-term, randomized, multicenter study that compared laparoscopic antireflux surgery with PPI treatment in patients with chronic GERD, all investigated histologic markers of microscopic esophagitis, i.e. basal cell layer hyperplasia, papillary elongation, intercellular space dilation, and intraepithelial eosinophils, significantly improved after 1 and 3 years, respectively, irrespective of the type of therapy applied [55].

Our study is well in the line with these literature data. The prevalence and severity of histologic findings, in particular those related to proliferative changes of the squamous epithelium, was inversely associated with the history of PPI intake.

This observation renders indirect evidence for the assumption that the histologic changes, following the Esohisto consensus guidelines for the diagnosis of microscopic esophagitis, are indeed related to abnormal esophageal acid exposure [16, 22, 52, 53].

Traditionally, the cardia has been considered as the most proximal part of the stomach, i.e. cardia ventriculi, and constitutes a natural buffer zone between the stomach and the esophagus to resist reflux of acid gastric content. A malignant growth in this region is called a carcinoma of the cardia and regarded as a gastric neoplasm [6, 56]. The point of origin and the significance of CM is still a contentious issue in the literature.

In 1997 [57] and 2000 [35], a first connection between the presence of CM at the GEJ and GERD was established. In a biopsy study performed on 71 patients, presence of CM and/or OCM between the squamous epithelium of the esophagus and the gastric mucosa (squamo-oxyntic gap) [58] was reported in all patients [35]. Further, patients with a squamo-oxyntic gap of more than 2 cm showed higher pH values in pH manometry than those with a length of less than 2 cm. This relation between the extent of CM and OCM and the amount of acid exposure in the distal esophagus allowed the authors to conclude, that the presence of CM and OCM can, as a sensitive histologic marker, predict the severity of GERD [35].

Several autopsy studies conducted on fetuses, children and adolescents, reported a presence of CM and OCM in every individual [59-61], hereby arguing that CM at the GEJ is a congenital histologic finding.

In a previous publication of the *histo*GERD trial [49] we found that CM was present in our study population in 713 (66.6%) individuals and was related to patients´ symptoms, body mass index, endoscopic diagnosis of esophagitis, as well as intestinal metaplasia and the histologic criteria of microscopic esophagitis according to the Esohisto project ($p < 0.001$). These findings, especially the association between CM and histologic lesions indicative of microscopic esophagitis, point to fact that CM seems to be a metaplastic lesion which probably arises from chronic acid reflux. Supporting our results, Glickman et al. [62], reported an association between the length of CM (pure mucous-type glands) and the diagnosis of active esophagitis in pediatric patients. Thereby suggesting, that

induced by chronic reflux, damage and recovery of the squamous epithelium result in an expansion of the squamo-oxyntic gap and development of a dilated distal esophagus [63]. Chandrasoma et al. [63] consider the “dilated GERD-damaged distal esophagus”, as an early event in GERD and therefore a prospective marker.

In conclusion, application of the Esohisto consensus guidelines was simple and proved feasible in all patients. In the *histo*GERD trial we systematically evaluated the histologic criteria presented in the Esohisto project, using a standardized biopsy protocol and related the prevalence and severity of microscopic lesions to various clinical variables indicative of GERD. Histologic criteria for diagnosis of esophagitis were significantly associated with patients’ symptoms, male gender, endoscopic diagnosis of esophagitis and history of PPI intake. Based upon these data, the Esohisto consensus guidelines proved to correlate with clinical characteristics and symptoms of GERD and demonstrate an additive value of histology in the work-up of patients with reflux disease.

Our findings support that histology, if systematically applied, may contribute important diagnostic clues to diagnose GERD in clinical practice. Therefore, we suggest that biopsies should routinely be obtained when patients undergo upper gastrointestinal endoscopy for evaluation of GERD and may particularly be beneficial in patients with NERD.

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Appendix

Patients´ questionnaire (in German)

Fragebogen zu Beschwerden im Oberbauch

(Bitte nicht ausfüllen)
Code:
Zentrum / PatientIn
...../.....

Name, Vorname(n): _____

Geburtsdatum: _____

1. Wie häufig traten bei Ihnen in den vergangenen vier Wochen folgende Beschwerden auf?

(Bitte kreuzen Sie für jede Beschwerde nur eine Antwortmöglichkeit an!)

	niemals → täglich				
Ein brennendes Gefühl hinter dem Brustbein	1	2	3	4	5
Schmerzen hinter dem Brustbein	1	2	3	4	5
Ein brennendes Gefühl im Zentrum des Oberbauches	1	2	3	4	5
Schmerzen im Zentrum des Oberbauches	1	2	3	4	5
Ein säuerlicher Geschmack im Mund	1	2	3	4	5
Ein unangenehmes Aufstoßen von Mageninhalt	1	2	3	4	5

2. Wie würden Sie die Beschwerden in den vergangenen vier Wochen bewerten?

(Bitte kreuzen Sie für jede Beschwerde nur eine Antwortmöglichkeit an!)

	Keine Beschwerden → Starke Beschwerden				
Ein brennendes Gefühl hinter dem Brustbein	1	2	3	4	5
Schmerzen hinter dem Brustbein	1	2	3	4	5
Ein brennendes Gefühl im Zentrum des Oberbauches	1	2	3	4	5
Schmerzen im Zentrum des Oberbauches	1	2	3	4	5
Ein säuerlicher Geschmack im Mund	1	2	3	4	5
Ein unangenehmes Aufstoßen von Mageninhalt	1	2	3	4	5

3. Abschließend wüssten wir gern, ob Sie rauchen – sind Sie?

☐ Raucher ☐ Ex-Raucher, seit wann: ..... ☐ Nichtraucher

Für Raucher und Ex-Raucher: ..... Zigaretten pro Tag seit/über ..... Jahr(e)

Assessment form for clinical data (in German)

(Bitte nicht ausfüllen)
Code:
Zentrum / PatientIn
...../.....

Klinisches Protokoll

Kenndaten

Name, Vorname(n):
Geschlecht ☐ männlich ☐ weiblich
Geburtsdatum:

Klinische Beschwerden / Indikation zur Endoskopie

Beschwerden	**Indikationen**
☐ Reflux	Endoskopie „wegen Ösophagus“
☐ Epigastrische Symptome	Endoskopie „wegen Magen“
☐ Diarrhö / Malabsorption	Endoskopie „wegen Duodenum“
☐ Andere: …….....	Andere: ……

(z.B. andere Beschwerden / Indikationen: Pat. betreffend GIT beschwerdefrei / Indikation vor Herz-OP)

Klinisch-anamnestische Daten

Größe:
Gewicht:
Medikamenten- und Alkoholanamnese:

PPI	☐ ja ☐ nein			
Falls ja:	☐ regelmäßig	☐ unregelmäßig		
seit wann:	Substanz:		Dosierung:	mg/d
Andere Säureblocker	☐ ja ☐ nein			
Falls ja:	☐ regelmäßig	☐ unregelmäßig		
seit wann:	Substanz:		Dosierung:	mg/d
NSAR	☐ ja ☐ nein			
Falls ja:	☐ regelmäßig	☐ unregelmäßig		
seit wann:	Substanz:		Dosierung:	mg/d
Alkohol	☐ ja ☐ nein			
Falls ja:	☐ regelmäßig	☐ unregelmäßig		
Menge:	…… g/die			

Assessment form for endoscopic data (in German)

Endoskopische Daten

(Bitte nicht ausfüllen)
Code:
Zentrum / PatientIn
...../.....

Magen

Gastritis ☐ ja ☐ nein

Falls ja: ☐ Typ A Gastritis
☐ Typ B Gastritis
☐ Typ C Gastritis
☐ nicht zuzuordnen

Intestinale Metaplasie ☐ ja ☐ nein

Ösophagus

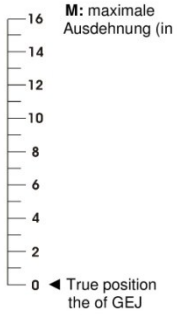
Hiatushernie ☐ ja ☐ nein

Los Angeles Klassifikation

Stadium		
N	☐	Normal mucosa
M	☐	Minimal changes to the mucosa, such as erythema and/or whitish turbidity
A	☐	Non-confluent mucosal breaks ≤ 5 mm in length
B	☐	Non-confluent mucosal breaks ≥ 5 mm in length
C	☐	Confluent mucosal breaks ≤ 75% circumferential
D	☐	Confluent mucosal breaks ≥ 75% circumferential

Prag Klassifikation bei Barrett-Ösophagus
(bitte mit Pfeilen markieren)

C: zirkuläres Segment (in cm)
cm)



M: maximale Ausdehnung (in

Bemerkungen (spezielle Befunde, z.B. Erosionen, Ulzera, Polypen):

Arzt / Ärztin: Name ..... Unterschrift .....

Assessment form for histologic data (in German)

(Bitte nicht ausfüllen)

Code:

Zentrum / PatientIn
..... /.....

Biopsieprotokoll

Kenndaten

Name, Vorname

Geburtsdatum

Teil 1

Biopsien aus der gastroösophagealen Übergangszone am proximalen Ende der Magenfalten (je 2PE seitengetrennt)

Pathologische Dokumentation je Probe	**Kl. Kurvatur**	**Gr. Kurvatur**
Plattenepithel	ja nein	ja nein
Falls ja		
Verbreiterte Basalzelllagen	0 - 1 - 2	0 - 1 - 2
Elongation der Papillen	0 - 1 - 2	0 - 1 - 2
Aufgeweitete Interzellularbrücken	0 - 1 - 2	0 - 1 - 2
Mononukleäre Zellen	0 - 1 - 2	0 - 1 - 2
Eosinophile Granulozyten	0 - 1 - 2	0 - 1 - 2
Neutrophile Granulozyten	0 - 1 - 2	0 - 1 - 2
Nekrose (Erosion / Ulkus)	ja nein	ja nein
Zylinderepithel	ja nein	ja nein
Drüsen vom Corpus bzw. Fundustyp (OM, oxyntic mucosa)	ja nein	ja nein
Drüsen vom Cardiatyp und Fundus/Corpustyp (OCM, oxyntocardiac)	ja nein	ja nein
Drüsen vom Cardiatyp (CM, cardiac mucosa)	ja nein	ja nein
Cardiagastritis (Sydney System)	ja nein	ja nein
Aktivität:	1 - 2 - 3	1 - 2 - 3
Chronizität:	1 - 2 - 3	1 - 2 - 3
Pankreatische Metaplasie	ja nein	ja nein
Intestinale Metaplasie	ja nein	ja nein
Ausprägung:	1 - 2 - 3	1 - 2 - 3
Typ:	I - II - III	I - II - III
LG Dysplasie (IEN)	ja nein	ja nein
HG Dysplasie (IEN)	ja nein	ja nein
Karzinom	ja nein	ja nein
Multilayered epithelium	ja nein	ja nein

Gesonderte Biopsate aus endoskopischer Barrett-Mukosa

Multilayered		BZ / Barrett		OM		OCM		CM	
ja	nein	ja	nein	ja	nein	ja	nein	ja	nein

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Original contribution

Validation study of the Esohisto consensus guidelines for the recognition of microscopic esophagitis (*histo*GERD Trial)☆

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Esophagitis;
Los Angeles classification;
Histology;
Pathology

Summary In patients with gastroesophageal reflux disease (GERD), histology is generally believed to be a tool of limited diagnostic value. Our study aimed to assess the prevalence of microscopic esophageal lesions as defined by the Esohisto consensus guidelines, which have proven high interobserver agreement in previous studies. In the prospective Central European multicenter *histo*GERD trial, we recruited 1071 individuals (576 females and 495 males; median age, 53 years; range, 15-93 years) undergoing gastroscopy for nonselected reasons. Biopsy material was systematically sampled from above and below the gastroesophageal junction. Overall, histologic diagnosis of mild and severe esophagitis was made in 423 (39.5%) and 296 (27.6%) individuals, respectively, whereas the squamous mucosa of 352 individuals (32.9%) was normal upon histology or showed only insignificant findings. Proliferative changes of the squamous epithelium, in particular basal cell layer hyperplasia, papillary elongation, and intercellular space dilation, were more common than inflammatory cell infiltration. The presence of microscopic esophagitis was associated with male sex ($P = .009$), patients' symptoms ($P = .003$), history of proton pump inhibitor intake ($P < .001$), and the endoscopic diagnosis of esophagitis ($P < .001$). Notably, among the 450 patients with no endoscopic signs of esophagitis (Los Angeles Category N), 41.8% and 17.1% were identified with mild and severe (microscopic) esophagitis, respectively, indicating higher sensitivity of histologic diagnosis. In conclusion, our data illustrate the

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value of histology in the workup of patients with reflux disease. We suggest that biopsies should routinely be obtained when patients undergo upper gastrointestinal endoscopy for evaluation of GERD and may particularly be beneficial in patients with nonerosive reflux disease.

1. Introduction

The prevalence of gastroesophageal reflux disease (GERD) ranges from 10% to 20% in the Western world when heartburn and/or acid regurgitation occurring at least once a week are used for definition. The prevalence in Asia is lower, accounting for less than 5% [1]. GERD comprises a wide variety of clinical manifestations, ranging from reflux symptoms without visible lesions at conventional endoscopy (ie, microscopic esophagitis) to grossly visible esophagitis with varying extent of mucosal breaks, graded according to the (modified) Los Angeles classification [2,3].

According to recently published practice guidelines of the American College of Gastroenterology, the diagnosis of GERD can be established in the setting of typical symptoms. Empiric medical therapy with a proton pump inhibitor (PPI) may be helpful in this setting (so-called PPI test). Upper endoscopy is not required in the presence of typical GERD symptoms, and routine biopsies from the distal esophagus are not recommended [4].

In patients with nonerosive reflux disease (NERD), histology is generally believed to be a tool of limited diagnostic value [5,6]. Several studies have, however, demonstrated that histology, if systematically applied, may render important diagnostic clues as at least two-thirds of NERD patients have histologic evidence of esophageal injury [7,8]. The Esohisto project is a multinational initiative for the standardized recognition of microscopic lesions in patients with GERD [9,10]. Histologic lesions evaluated were basal cell layer hyperplasia, papillary elongation, intraepithelial eosinophil, neutrophil, and mononuclear cell number, as well as necrosis/erosion, healed erosion, and dilation of intercellular spaces. The project has proven good interobserver agreement (ranging from 64% to 97%). However, a validation study, correlating the evaluated microscopic features with clinical variables, such as reflux symptoms and endoscopic features, has not been performed yet.

In the prospective Central European multicenter *histo*-GERD trial, we recruited 1130 individuals undergoing gastroscopy for various nonselected reasons. Biopsy material was systematically sampled from the gastroesophageal junction (GEJ). In this study, we aimed to assess the prevalence of microscopic esophageal lesions, as defined in the Esohisto consensus guidelines. Specifically, we related their presence to various clinical and/or endoscopic features indicative of GERD, thereby evaluating the clinical significance of the Esohisto project.

2. Materials and methods

We conducted a prospective cross-sectional study to assess the prevalence of microscopic esophageal lesions in patients with GERD and to evaluate associations between histologic and clinical data including endoscopic findings. Data will be presented following the STROBE Statement aimed at strengthening the reporting of observational studies [11].

2.1. Study population

Participants were prospectively recruited in the multicenter central European *histo*GERD trial that aimed at systematically investigating clinical, particularly endoscopic data and histologic findings in individuals with and without symptoms of reflux disease who underwent endoscopic evaluation of their upper gastrointestinal tract.

In Austria, 3 clinical departments (Department of Internal Medicine, Krankenhaus der Barmherzigen Brüder, St Veit/Glan, Department of Surgery, Division of General Surgery, and Department of Internal Medicine, Division of Gastroenterology and Hepatology, Medical University of Graz, Graz, Austria) and, in Germany, 2 private practices (Dr M. Geppert and Dr B. Schmack, Bayreuth, and Dr H. Bordel, Dr R. Müller, and Dr B. Wigginghaus, Osnabrück) participated in the investigation.

During the study period, adult men and women scheduled for elective endoscopic examination for unselected reasons were offered participation. We excluded those with previous surgery leading to abnormal anatomy in the upper gastrointestinal tract, particularly at the GEJ. In Austria, patients were recruited between November 2011 and April 2012, in Germany between December 2011 and May 2012, respectively.

The investigation was carried out in accordance with the Declaration of Helsinki. Each participant provided written informed consent. The study was approved by the Institutional Review Boards of the Medical University of Graz (EK 24–052 ex 11/12) and the University of Erlangen (EK 4571 ex 11/12), respectively, and was registered at ClinicalTrials.gov (NCT01576289).

2.2. Endoscopy

The esophagus, GEJ, and stomach of all participants were examined according to a standardized protocol devised for the study. The *GEJ* was defined as the most proximal extent

Table 1 Histologic criteria for the recognition and assessment of microscopic lesions related to GERD–the Esohisto project [9,10]

Criterion	Definition and method of assessment	Severity score
Basal cell layer hyperplasia	Measure basal cell layer thickness in micrometers and express as a proportion (%) of total epithelial thickness (×10)	0 (<15%), 1 (15%-30%), 2 (>30%)
Papillary elongation	Measure papillary length in micrometers and express as a proportion (%) of total epithelial thickness (×10)	0 (<50%), 1 (50%-75%), 2 (>75%)
Dilation of intercellular spaces	Identify as irregular round dilations or diffuse widening of intercellular space (×40)	0 (absent), 1 (small; diameter <1 lymphocyte), 2 (large; diameter ≥1 lymphocyte)
Intraepithelial eosinophils	Count in the most affected high-power field (×40)	0 (absent), 1 (1-2 cells), 2 (>2 cells)
Intraepithelial neutrophils	Count in the most affected high-power field (×40)	0 (absent), 1 (1-2 cells), 2 (>2 cells)
Intraepithelial mononuclear cells	Count in the most affected high-power field (×40)	0 (0-9 cells), 1 (10-30 cells), 2 (>30 cells)

of the gastric folds, that is, the point where the tubular esophagus meets the gastric folds.

Before the procedure, the endoscopists recorded basic demographic data including patient age and sex and classified the indication for endoscopy as follows: (i) patients evaluated for diseases of the esophagus reporting heartburn, acid regurgitation, or both at least once a week (according to Lagergren's criteria for GERD [12]) and/or dysphagia, (ii) patients evaluated for diseases of the stomach reporting epigastric discomfort and/or pain, and (iii) patients evaluated for diseases of the small bowel reporting diarrhea (with or without weight loss), with multiple answers possible. During the procedure, the endoscopists graded the severity of esophagitis according to the modified Los Angeles classification, which focuses on the extent of mucosal breaks, but also refers to minimal changes [2,3].

Biopsies were systematically taken from the GEJ as follows: At least 2 specimens each were obtained from the most proximal extent of the gastric folds (greater and lesser curvature) and from the distal esophagus with the aim of obtaining tissue samples from both above and below the squamocolumnar junction. Specifically, squamous epithelium was sampled within 1 cm above the squamocolumnar junction. However, when columnar metaplasia was observed in the distal esophagus, the biopsies were obtained upwards until squamous epithelium was reached. Thus, a minimum of 4 biopsies was obtained from each patient, each biopsy fragment of tissue measuring approximately 2 mm in greatest diameter.

All endoscopists were very experienced in the field: most of them had worked in endoscopy units for at least 1 decade performing more than 500 gastroscopies per year. Before the investigation, all endoscopists were trained to familiarize them with the biopsy protocol and, particularly, the reporting system. For endoscopy, 4 institutions used the OLYMPUS EVIS EXERA II series (Olympus Europe Holding GmbH, Hamburg, Germany) with video gastroscopes GIF-H180 and Q180, respectively. One institution (Osnabrück) used the FUJI EPX-4450HD Electronic Video Endoscopy System with EG-590WR video gastroscopes (Fujifilm Corporation, Tokyo, Japan).

2.3. Histopathology

Biopsy specimens were fixed in 4% buffered formalin, embedded in paraffin, cut at 4 to 5 levels as 2-μm-thick sections and stained with hematoxylin and eosin for histologic examination. All biopsies were assessed by 2 experienced gastrointestinal pathologists (M. V. and C. L.) who were blinded to clinical data including endoscopic findings.

Esophageal biopsies obtained proximal to the squamocolumnar junction were examined for the presence or absence

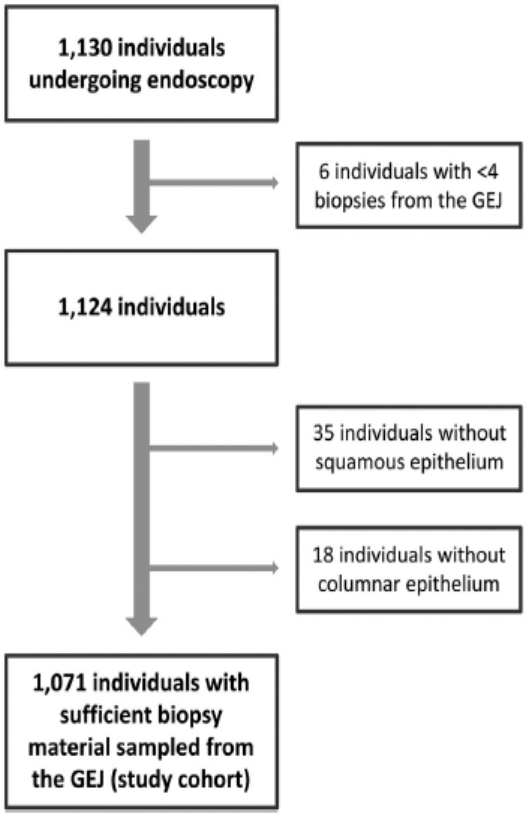

Fig. 1 Study cohort for the evaluation of microscopic esophagitis (*histo*GERD trial).

of microscopic esophageal lesions according to recently published consensus guidelines (Esohisto project) [9,10]. These guidelines provide a systematic basis to score the extent of various histologic features, such as basal cell layer hyperplasia, papillary elongation, dilation of intercellular spaces, and the presence of intraepithelial inflammatory cells. Details are presented in Table 1.

In addition to assessing the severity of individual lesions (score range, 0-2), a combined severity score was also obtained for each patient by summing up lesion scores and dividing by the number of lesion types assessed. As suggested in the Esohisto project, the calculation was restricted to basal cell layer hyperplasia, papillary elongation, dilation of intercellular spaces, and the presence of intraepithelial eosinophils as these are the most informative elementary lesions. Scores 0 to 0.25 were regarded as normal, scores 0.5 to 0.75 qualified for diagnosis of "mild" esophagitis, and scores of 1 or higher qualified for diagnosis of "severe" esophagitis [10,13].

2.4. Statistical analysis

All data were included within a prospective joint database. The quality of this database was tested by random sample taking in 5% of the recruited patients, which revealed misreporting in 0.14%. Categorical variables are presented as

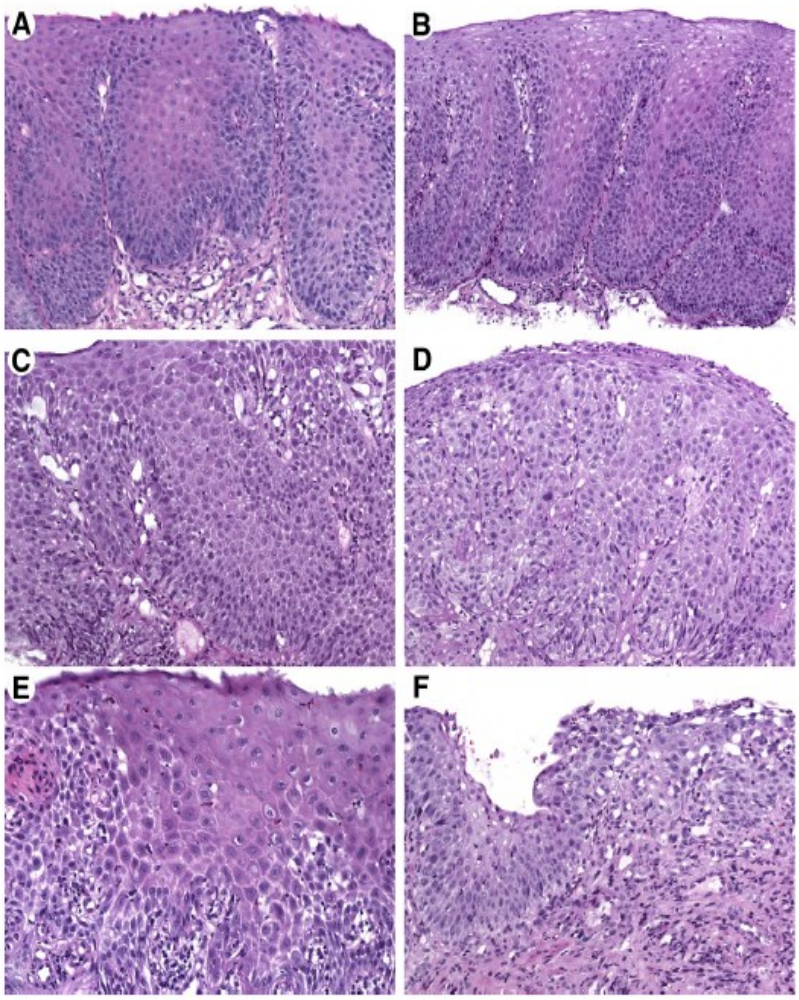

Fig. 2 Examples of microscopic esophagitis in patients with GERD: A, 38-year-old woman with mild esophagitis (basal cell layer hyperplasia score 1, papillary elongation score 2; combined severity score, 0.75). B, 26-year-old woman with mild esophagitis (basal cell layer hyperplasia score 1, papillary elongation score 1, intercellular space dilation score 1; combined severity score, 0.75). C, 26-year-old man with severe esophagitis (basal cell layer hyperplasia score 2, papillary elongation score 2, intercellular space dilation score 1; combined severity score, 1.25). D, 58-year-old man with severe esophagitis (basal cell layer hyperplasia score 2, papillary elongation score 2, intercellular space dilation score 2; combined severity score, 1.5). E, 42-year-old man with severe esophagitis (basal cell layer hyperplasia score 1, papillary elongation score 2, intercellular space dilation score 2, intraepithelial eosinophils score 2; combined severity score, 1.75). F, 42-year-old man with severe esophagitis next to an active erosion (basal cell layer hyperplasia score 2, papillary elongation score 2, intercellular space dilation score 2, intraepithelial eosinophils score 2; combined severity score, 2).

absolute and relative frequencies; numerical variables, as medians and ranges as well as means. Differences in categorical variables were examined using the χ^2 test or Fisher exact test, as appropriate. Differences in continuous variables between groups were analyzed using the Mann-Whitney *U* test. All statistical calculations were performed using NCSS [14]. Two-sided *P* values less than .05 were considered statistically significant.

3. Results

3.1. Patient characteristics

In all, 1130 individuals participated in the study. Six individuals who did not reach the minimum of 4 biopsies sampled from the GEJ as well as 35 individuals without squamous and 18 individuals without columnar epithelium sampled from the GEJ were excluded from analysis, respectively, leading to a final study cohort of 1071 participants (94.8%) (Fig. 1).

There were 576 females (53.8%) and 495 males (46.2%) (ratio, 1.16:1). Median age was 53 years (mean, 52.1; range, 15-93). In 142 individuals (13.3%), no medical indication for gastroscopy was provided. When the medical indications of the remaining individuals were stratified, 452 (42.2%) were evaluated for diseases of the esophagus; 623 (58.2%), for diseases of the stomach; and 155 (14.5%), for diseases of the small bowel. In 180 individuals (16.8%), endoscopists referred to "other" indications, such as preoperative endoscopy (eg, before cholecystectomy or bariatric surgery), evaluation of individuals with inflammatory bowel disease for upper gastrointestinal tract involvement and evaluation of individuals with anemia, weight loss, and metastatic disease with unknown primary.

At time of investigation, 503 participants (47%) were on PPIs, with 330 (65.6%) receiving PPIs on a regular basis. For 157 of these, data regarding the duration of PPI intake were available, with mean and median values accounting for 31.4 and 12 months, respectively.

3.2. Histologic diagnosis of esophagitis

Applying the combined severity score for the diagnosis of microscopic esophageal lesions, the squamous mucosa was normal upon histology (or showed only insignificant findings) in 352 individuals (32.9%), was diagnosed with mild esophagitis in 423 individuals (39.5%), or was diagnosed with severe esophagitis in 296 individuals (27.6%) (Fig. 2). Median age of individuals with normal histology, mild esophagitis, and severe esophagitis was 55 (mean, 53.9; range, 15-91), 53 (mean, 52.2; range, 15-93), and 50 (mean, 49.7; range, 15-85) years, respectively. Prevalence and severity of esophagitis were significantly associated with sex, as 42.3% of individuals with normal histology were males, compared with 44.2% with mild and 53.7% with severe esophagitis ($P = .009$).

The microscopic diagnosis of esophagitis was significantly associated with patients' symptoms. This holds true for the combined severity score as well as the following elementary lesions: basal cell layer hyperplasia, papillary elongation, and presence of intraepithelial neutrophils. Details are presented in Table 2. In addition, prevalence and severity of histologic findings were inversely associated with PPI intake. Thus, patients receiving PPIs, either on demand or on a regular basis, showed significantly milder histologic changes (Table 3).

3.3. Relation between histologic and endoscopic diagnosis of esophagitis

The prevalence and severity of esophagitis were assessed according to the modified Los Angeles classification as follows: 450 (42%) were normal (Category N) upon endoscopic inspection, 303 (28.3%) individuals presented with minimal changes (Category M), and 318 (29.7%) presented with mucosal breaks of different extent (Categories A-D; Fig. 3). The endoscopic diagnosis of esophagitis

Table 2 Histologic criteria for the recognition and assessment of microscopic lesions (and combined severity score for the diagnosis of esophagitis according to Esohisto guidelines) related to the presence of symptoms indicating esophageal disease, such as heartburn, acid regurgitation, or both at least once a week while not consuming antireflux medication and/or dysphagia

Criterion	Severity score	Symptoms of esophageal disease		*P*
		Absent (n = 619)	Present (n = 452)	
Basal cell layer hyperplasia	0	208 (33.6%)	119 (26.3%)	.012
	1	281 (45.4%)	210 (46.5%)	
	2	130 (21%)	123 (27.2%)	
Papillary elongation	0	228 (36.8%)	128 (28.3%)	.0019
	1	271 (43.8%)	202 (44.7%)	
	2	120 (19.4%)	122 (27%)	
Dilation of intercellular spaces	0	292 (47.2%)	191 (42.3%)	.28
	1	265 (42.8%)	211 (46.7%)	
	2	62 (10%)	50 (11.1%)	
Intraepithelial eosinophils	0	568 (91.8%)	411 (90.9%)	.77
	1	27 (4.4%)	24 (5.3%)	
	2	24 (3.9%)	17 (3.8%)	
Intraepithelial neutrophils	0	601 (97.1%)	428 (94.7%)	.047
	1	5 (0.8%)	12 (2.7%)	
	2	13 (2.1%)	12 (2.7%)	
Intraepithelial mononuclear cells	0	341 (55.1%)	220 (48.7%)	.11
	1	253 (40.9%)	210 (46.5%)	
	2	25 (4%)	22 (4.9%)	
Combined severity score	Normal	228 (36.8%)	124 (27.4%)	.003
	Mild	237 (38.3%)	186 (41.2%)	
	Severe	154 (24.9%)	142 (31.4%)	

Table 3 Histologic criteria for the recognition and assessment of microscopic lesions (and combined severity score for the diagnosis of esophagitis according to Esohisto guidelines) related to intake of PPIs

Criterion	Severity score	PPI intake			*P*
		No PPI (n = 568)	Irregular (n = 173)	Regular (n = 330)	
Basal cell layer hyperplasia	0	146 (25.7%)	59 (34.1%)	122 (37%)	.001
	1	268 (47.2%)	72 (41.6%)	151 (45.8%)	
	2	154 (27.1%)	42 (24.3%)	57 (17.3%)	
Papillary elongation	0	155 (27.3%)	63 (36.4%)	138 (41.8%)	<.001
	1	267 (47%)	69 (39.9%)	137 (41.5%)	
	2	146 (25.7%)	41 (23.7%)	55 (16.7%)	
Dilation of intercellular spaces	0	231 (40.7%)	84 (48.6%)	168 (50.9%)	<.001
	1	268 (47.2%)	69 (39.9%)	139 (42.1%)	
	2	69 (12.1%)	20 (11.6%)	23 (7%)	
Intraepithelial eosinophils	0	514 (90.5%)	158 (91.3%)	307 (93%)	.086
	1	34 (6%)	4 (2.3%)	13 (3.9%)	
	2	20 (3.5%)	11 (6.4%)	10 (3%)	
Intraepithelial neutrophils	0	544 (95.8%)	164 (94.8%)	321 (97.2%)	.15
	1	13 (2.3%)	2 (1.2%)	2 (0.6%)	
	2	11 (1.9%)	7 (4%)	7 (2.1%)	
Intraepithelial mononuclear cells	0	270 (47.5%)	95 (54.9%)	196 (59.4%)	.005
	1	266 (46.8%)	71 (41%)	126 (38.2%)	
	2	32 (5.6%)	7 (4%)	8 (2.4%)	
Combined severity score	Normal	156 (27.5%)	63 (36.4%)	133 (40.3%)	<.001
	Mild	238 (41.9%)	57 (32.9%)	128 (38.8%)	
	Severe	174 (30.6%)	53 (30.6%)	69 (20.9%)	

was significantly associated with patients' symptoms: 136 individuals (30.2%) with normal mucosa (Category N), 140 individuals (46.2%) with minimal changes (Category M), and 176 individuals (55.3%) with mucosal breaks (Category A-D) reported on heartburn, acid regurgitation, and/or dysphagia ($P < .001$). When the histologic diagnosis of both elementary lesions and combined severity score was related to the endoscopic diagnosis of esophagitis, a highly significant association was identified. Details are presented in Table 4.

4. Discussion

Various histologic features of the squamous epithelium related to GERD have been identified. These include basal cell layer hyperplasia and papillary elongation [8,15], presence of intraepithelial neutrophils [16], eosinophils [17,18], and mononuclear cells [19,20]. More recently, dilation of intercellular spaces has been identified as another important feature [21–24].

The consensus guidelines presented by the Esohisto project provide an excellent basis for standardized recognition of microscopic esophagitis, including the grading of disease severity. In particular, the guidelines refer to a severity score for the tested individual lesions (ranging from 0 to 2) [9]. In a subsequent article, aimed at the refinement and reproducibility of histologic criteria, the authors introduced a combined severity score restricted to basal cell layer hyperplasia, papillary elongation, dilation of intercellular spaces, and the presence of intraepithelial eosinophils [10] as these 4 features are considered to be the most informative elementary lesions [13]. Levels of interobserver agreement were tested for all features. The percentage agreement was 64% for dilation of intercellular spaces and was in the range 73% to 74% for basal cell layer hyperplasia, papillary elongation, and intraepithelial mononuclear cells and 83% to 97% for intraepithelial eosinophils, intraepithelial neutrophils, active erosions, and healed erosions [10].

In the *histo*GERD trial, we prospectively evaluated the histologic features presented in the Esohisto project, using a standardized biopsy protocol. Specifically, we related the prevalence and severity of microscopic lesions to various clinical and endoscopic features indicative of GERD. The definitions provided in the Esohisto consensus guidelines are simple and proved feasible in all patients. According to our study, the histologic features for diagnosis of esophagitis were significantly associated with patients' symptoms and the endoscopic diagnosis of esophagitis. Thus, in contrast to previous reports on the marginal role of histology in patients with GERD [5,6], our data clearly indicate that, although not routinely recommended in current practice guidelines [4,25], histology can well serve as a useful diagnostic tool.

In the recent study by Zentilin et al [7], similar results were presented: the sensitivity of histology was 96% in patients with erosive esophagitis, whereas among NERD patients, histologic abnormalities were found in 47 (80%) of 59 cases with abnormal pH testing and in 7 (58%) of 12 cases

with negative pH testing. Comparable results were obtained in our study: among the 450 patients with no endoscopic signs of esophagitis (Los Angeles Category N), 41.8% and 17.1% were identified with mild and severe (microscopic) esophagitis, respectively, indicating higher sensitivity of histologic diagnosis. As in our investigation, proliferative changes of the squamous epithelium, in particular, basal cell layer hyperplasia and dilation of intercellular spaces, were the most frequent abnormalities in the study by Zentilin et al [7]. Intraepithelial eosinophils were more common than neutrophils. Despite their high prevalence, the diagnostic impact of eosinophils was poor because they did not influence the specificity of histology in controls and their presence influenced the histologic classification in only 2 patients (normal versus pathologic). The authors concluded that the diagnostic role of histology in patients with GERD should be reconsidered, as it is able to provide useful and objective additional data in 76% of patients with NERD.

In our investigation, infiltration of the squamous epithelium by mononuclear cells was found to be more frequent than infiltration by eosinophils or neutrophils. This observation is well in the line with the ProGERD study [26]. Comparable to that study, the infiltration by eosinophils and neutrophils markedly increased with increasing severity of endoscopic diagnosis of esophagitis in our study. In contrast, the increase in mononuclear cells was rather modest (Los Angeles N-M 45.2% versus Los Angeles A-D 53.5%), suggesting low sensitivity of this marker for the diagnosis of GERD.

In the ProGERD study, proliferative changes of the squamous epithelium, in particular, basal cell layer hyperplasia and papillary elongation, were associated with severity of disease. After PPI therapy, both thickness of basal cell layers and length of papillae were significantly reduced [27]. Likewise, complete recovery of dilated intercellular spaces, accompanied by regression of clinical symptoms, was reported in 92.1% and 97.4% of patients after 3 and 6 months of PPI therapy, respectively [28]. In the LOTUS trial, a long-term, randomized, multicenter study that compared laparascopic antireflux surgery with PPI treatment in patients with chronic GERD, all investigated histologic markers of microscopic esophagitis, that is, basal cell layer hyperplasia, papillary elongation, intercellular space dilation, and intraepithelial eosinophils, significantly improved after 1 and 3 years, respectively, irrespective of the type of therapy applied [29].

Our study is well in the line with these literature data. The prevalence and severity of histologic findings, in particular those related to proliferative changes of the squamous epithelium, were inversely associated with the history of PPI intake. This observation renders indirect evidence for the assumption that the histologic changes, following the Esohisto consensus guidelines for the diagnosis of microscopic esophagitis, are indeed related to abnormal esophageal acid exposure [8].

Our study has strengths and limitations. Apart from the prospective design and the high number of participants,

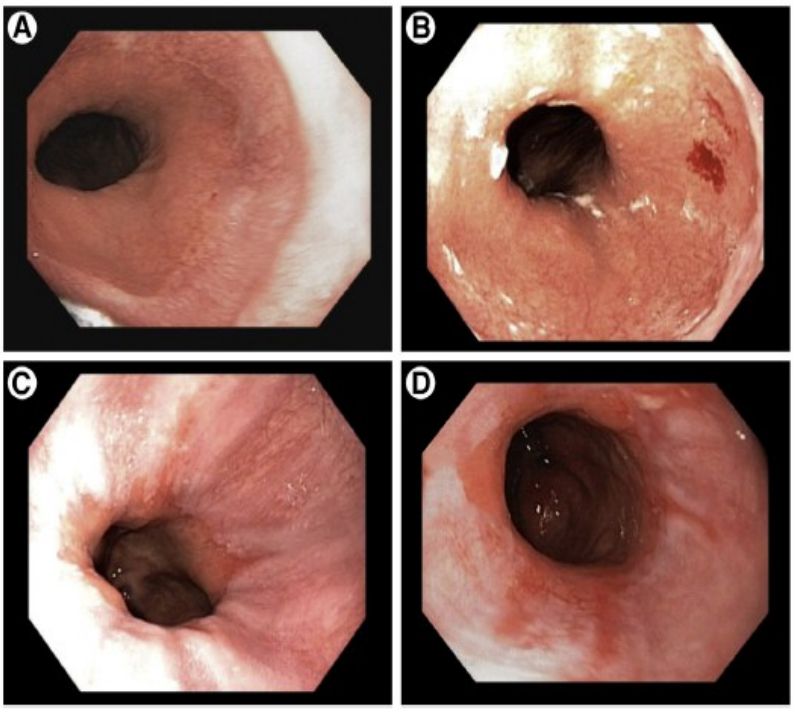

Fig. 3 Examples of endoscopic diagnoses of reflux esophagitis graded according to the modified Los Angeles classification: A, 21-year-old woman with normal GEJ (Los Angeles category N). B, 72-year-old man with minimal changes (Los Angeles category M). C, 55-year-old man with Los Angeles category A esophagitis. D, 63-year-old man with Los Angeles category B esophagitis.

Table 4 Histologic criteria for the recognition and assessment of microscopic lesions (and combined severity score for the diagnosis of esophagitis according to Esohisto guidelines) related to the endoscopic diagnosis of esophagitis, graded according to the modified Los Angeles classification [2,3]

Criterion	Severity score	Endoscopic diagnosis of esophagitis						*P*
		N (n = 450)	M (n = 303)	A (n = 190)	B (n = 110)	C (n = 10)	D (n = 8)	
Basal cell layer hyperplasia	0	170 (37.8%)	93 (30.7%)	40 (21%)	17 (15.5%)	4 (40%)	3 (37.5%)	<.001
	1	221 (49.1%)	141 (46.5%)	86 (45.3%)	38 (34.5%)	2 (20%)	3 (37.5%)	
	2	59 (13.1%)	69 (22.8%)	64 (33.7%)	55 (50%)	4 (40%)	2 (25%)	
Papillary elongation	0	186 (41.3%)	100 (33%)	46 (24.2%)	17 (15.5%)	4 (40%)	3 (37.5%)	.027
	1	203 (45.1%)	141 (246.5%)	83 (43.7%)	41 (37.3%)	2 (20%)	3 (37.5%)	
	2	61 (13.6%)	62 (20.5%)	61 (32.1%)	52 (47.3%)	4 (40%)	2 (25%)	
Dilation of intercellular spaces	0	229 (50.9%)	148 (48.8%)	68 (35.8%)	29 (26.4%)	5 (50%)	4 (50%)	<.001
	1	195 (43.3%)	135 (44.6%)	88 (46.3%)	52 (47.3%)	4 (40%)	2 (25%)	
	2	26 (5.8%)	20 (6.6%)	34 (17.9%)	29 (26.4%)	1 (10%)	2 (25%)	
Intraepithelial eosinophils	0	423 (94%)	283 (93.4%)	172 (90.5%)	86 (78.2%)	9 (90%)	6 (75%)	<.001
	1	9 (2%)	9 (3%)	16 (8.4%)	15 (13.6%)	1 (10%)	1 (12.5%)	
	2	18 (4%)	11 (3.6%)	2 (1.1%)	9 (8.2%)	0 (0%)	1 (12.5%)	
Intraepithelial neutrophils	0	444 (98.7%)	294 (97%)	182 (95.8%)	94 (85.5%)	10 (100%)	5 (50%)	<.001
	1	0 (0%)	2 (0.7%)	6 (3.1%)	7 (6.4%)	0 (0%)	2 (25%)	
	2	6 (1.3%)	7 (2.3%)	2 (1.1%)	9 (8.2%)	0 (0%)	1 (12.5%)	
Intraepithelial mononuclear cells	0	251 (55.8%)	162 (53.5%)	98 (51.6%)	42 (38.2%)	4 (40%)	4 (50%)	.003
	1	186 (41.3%)	128 (42.2%)	86 (45.3%)	55 (50%)	5 (50%)	3 (37.5%)	
	2	13 (2.9%)	13 (4.3%)	6 (3.1%)	13 (11.8%)	1 (10%)	1 (12.5%)	
Combined severity score	Normal	185 (41.1%)	99 (32.7%)	45 (23.7%)	16 (14.5%)	4 (40%)	3 (37.5%)	<.001
	Mild	188 (41.8%)	125 (41.3%)	73 (38.4%)	33 (30%)	2 (20%)	2 (25%)	
	Severe	77 (17.1%)	79 (26.1%)	72 (37.9%)	61 (55.5%)	4 (40%)	3 (37.5%)	

strengths include the detailed ascertainment of patients' complaints and endoscopic and histologic data. The interobserver bias of histologic examination was minimized as only 2 pathologists assessed the slides, but interobserver variation was beyond the scope of our investigation and was not specifically evaluated. These 2 pathologists were very experienced in the field, and before the study, they had discussed each histologic parameter thoroughly and agreed upon histology reporting. Nevertheless, our study has some limitations. Despite the fact that all endoscopists received detailed training and introduction to the biopsy protocol and to the classification of esophagitis, we cannot exclude interobserver bias among the various endoscopists, and interobserver variation was not specifically evaluated. In addition, this is a multicenter study, and although almost every institution used the same brand and model of endoscope, we cannot assure that the investigators made the endoscopic diagnosis of esophagitis in the same manner. Owing to the large number of study participants, no objective assessment of acid exposure to the esophagus, particularly 24-hour pH monitoring, was performed.

In conclusion, application of the Esohisto consensus guidelines was simple and proved feasible in all patients. Diagnosis of microscopic esophagitis was significantly associated with patients' symptoms, endoscopic diagnosis of esophagitis, and history of PPI intake, illustrating the value of histology in the workup of patients with reflux disease. We suggest that biopsies should routinely be obtained when patients undergo upper gastrointestinal endoscopy for evaluation of GERD and may particularly be beneficial in patients with NERD.

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