Original research article

The added value of magnifying endoscopy in diagnosing patients with certain gastroesophageal reflux disease

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ABSTRACT

Purpose: In most cases gastroesophageal reflux disease proceeds without macroscopic erosions in the esophagus. We aimed to clarify if abnormalities detectable in magnifying endoscopy may offer additional diagnostic criteria for gastroesophageal reflux disease and to what histopathologic structures do they correspond.

Patients/methods: Esophageal mucosa above and below Z-line was evaluated under x115 magnification in 67 gastroesophageal reflux disease patients (11 with erosive reflux disease, 28 with Barrett's esophagus, 28 with nonerosive reflux disease) and in 12 patients without gastroesophageal reflux disease (negative control group). Features characteristic of gastroesophageal reflux disease were specified by comparing erosive reflux disease and Barrett's esophagus patients with negative control group. Afterwards the presence of identified features were evaluated in nonerosive reflux disease group. Interobserver agreement in the recognition of the proposed criteria was rated. Biopsies collected from the mucosa above Z-line were evaluated histologically after hematoxylin and eosin staining.

Results: Endoscopic lesions characteristic of gastroesophageal reflux disease were: microerosions, abnormal intrapapillary capillary loops, obscured palisade vessels, white points, big triangular indentations of Z-line and villous mucosa below Z-line. The presence of two or more of the above features indicated gastroesophageal reflux disease with 97% sensitivity and 75% specificity. Substantial interobserver agreement was achieved in evaluation of obscured palisade vessels, abnormal intrapapillary capillary loops and white points. Endoscopic lesions were correlated to histology. Lesions identified with magnifying endoscopy were helpful in discerning between negative control group and nonerosive reflux disease patients.

Conclusions: Magnifying endoscopy reveals abnormalities that can be used as additional endoscopic diagnostic criteria of gastroesophageal reflux disease.

1. Introduction

Gastroesophageal reflux disease (GERD) is an increasing problem with approximately 20% of Western countries population suffering nowadays from its symptoms [1]. GERD is the most frequent diagnosis in gastroenterological outpatient clinics and causes a great burden to health resources [2]. GERD is also among the strongest known risk factors for esophageal adenocarcinoma which incidence has risen 6 fold during the last 40 years [3]. Diagnosis of GERD can be made based on typical symptoms and positive response to anti-secretory treatment [4]. Indications for further testing include treatment failure, diagnostic uncertainty and treating or preventing complications. Ambulatory

reflux monitoring like 24-hour pH-metry or pH-impedance can provide confirmatory evidence of GERD [5]. However, in many countries pH monitoring is less available than endoscopy, especially outside the reference and academic centers. GERD continues to be a common indication for upper gastrointestinal endoscopy reaching 24% of all procedures in the Unites States [2]. Based on the result of endoscopy, GERD can be divided into three types: Barrett's esophagus (BE), erosive reflux disease (ERD) and nonerosive reflux disease (NERD). BE and ERD have typical appearance in standard endoscopy but in case of NERD, which constitutes up to 65% of all patients, no lesions are visible [6]. Therefore, conventional endoscopy has low sensitivity as a diagnostic tool for GERD. Methods improving ability of endoscopy to detect GERD
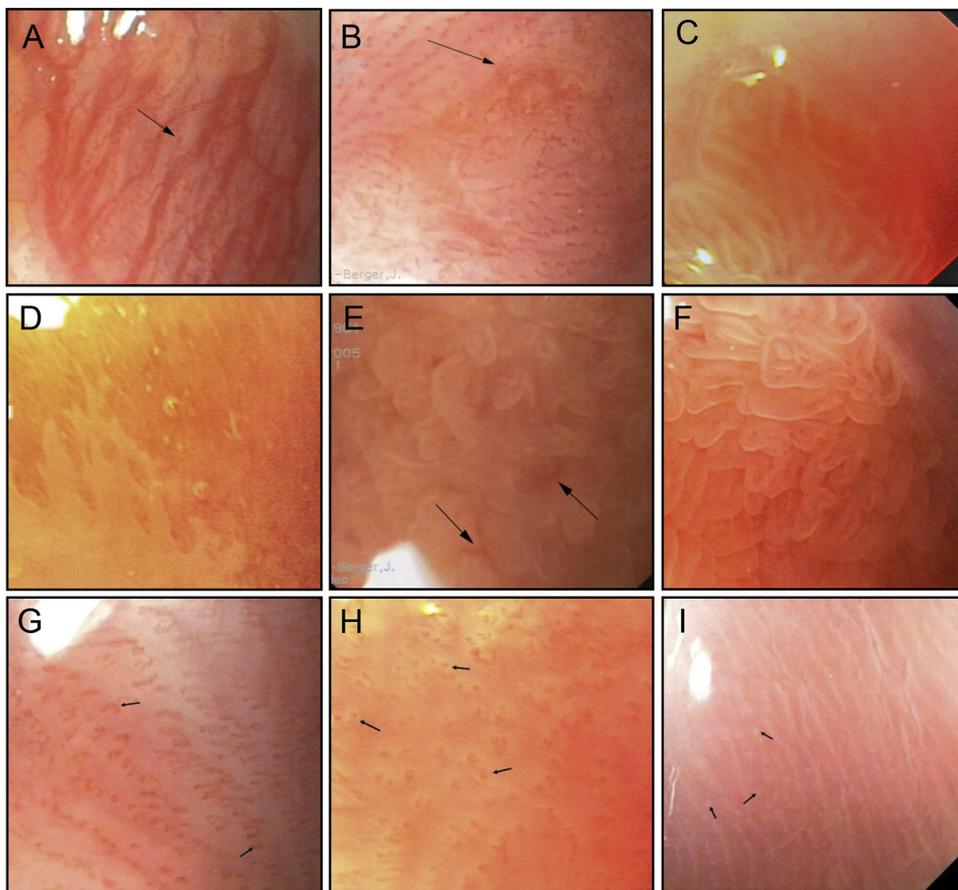
Fig. 1. (a) Normal squamous mucosa. Palisade vessels transparent, few intrapapillary capillary loops visible as dots (arrow). (b) Microerosion (arrow). (c) Big triangular indentation of Z-line. (d) Serrated Z-line. (e) Blood vessels transparent through columnar mucosa (arrows). (f) Villiform columnar mucosa below Z-line. (g) Abnormal intrapapillary capillary loops within squamous mucosa - increased in number, elongated, dilated (arrows). Palisade vessels obscured. (h) White points visible as whitish halation encircling intrapapillary capillary loops (arrows). Palisade vessels obscured. (i) White points visible as pinpoint spots in the absence of apparent intrapapillary capillary loops (arrows). Palisade vessels obscured.

are awaited.

One of advanced techniques developed for better evaluation of gastrointestinal tract is magnifying endoscopy (ME). The system of lenses inside the endoscope allow for optical magnification of the image and therefore detailed evaluation of mucosal surface and blood vessels. Because the degree of magnification is operator dependent, it is possible to obtain both standard and magnified images during the same endoscopic procedure.

Normal squamous mucosa of distal esophagus is transparent for longitudinal palisade vessels located in submucosa. At a magnification of x90, apart from the palisade vessels, regularly arranged, sparse, small pin-point like intrapapillary capillary loops (IPCLs) of lamina propria mucosae are visible [7] (Fig. 1a). The border between squamous and columnar epithelium, so-called Z-line is even, located at the upper end of gastric folds. Distally to the Z-line no vessels are seen and the surface of mucosa has regular dot-like pit pattern [8,9].

Research on novel endoscopic methods described a variety of minimal changes in NERD patients such as minute apical mucosal breaks, triangular indentations or serrated Z-line, obscured palisade vessels, enlarged IPCLs, villiform mucosa and transparent vessels below the Z-line [9,10,11] Most of the previously published studies on so-called minimal change esophagitis are of preliminary character and reproducibility of their findings still requires confirmation. Defining easy to detect features of GERD may increase the importance of endoscopy in NERD evaluation and reduce the demand for pH-metry.

Histological changes in GERD include elongation of the mucosal papillae, basal cell hyperplasia, inflammatory cells infiltration and dilated intercellular spaces [12,13]. Such lesions are also detected in NERD and acid-suppressing therapy reverses them [14]. However some studies point out low sensitivity and specificity of those findings [15].

The aim of our study was to evaluate which minimal lesions visible in ME in patients with features of GERD detected with conventional endoscopy are most characteristic and may serve as additional diagnostic criteria. We studied interobserver agreement for the presence of these minimal lesions. For better understanding of endoscopic morphological abnormalities, we searched for their histologic equivalents. Finally, we assessed the presence of identified minimal lesions in patients with NERD.

2. Patients and methods

2.1. Patients

We enrolled prospectively patients diagnosed with GERD on the basis of typical symptoms and positive response to proton pump inhibitor (PPI) treatment. Before inclusion, the patients with symptoms suggestive of GERD had two visits in our ambulatory clinic. During the first visit patients not treated with PPI were evaluated according to a questionnaire developed by Carlsson et al. [16] with a cut-off point for diagnosis of GERD at score 4 and were prescribed double standard dose of PPI (20 mg of omeprazole or 40 mg of pantoprazole twice a day) for 2 weeks. During the second visit patients with positive response to the prescribed treatment were diagnosed with GERD, qualified to the study (moment of inclusion) and referred for gastroscopy. The time between inclusion and endoscopy was up to 3 months. During that time patients were allowed to take PPI on demand but had to stop this treatment a week before endoscopy. We also included patients with previously diagnosed BE who were referred for upper gastrointestinal endoscopy as a periodic endoscopic screening for neoplasia. These patients were on chronic treatment with standard dose of PPI once daily or twice daily depending on subjective symptoms with 1-week off PPI before endoscopy. One week off PPI served as unification of the examined group with the negative control group, which did not take PPI at all and unification of the examined group itself, because particular GERD

patients could otherwise use more or less PPI because of their subjective symptoms.

Patients subjected to endoscopy were assigned after the procedure to one of the three groups: BE, ERD or NERD. Because NERD patients may be a heterogenous group including some patients with heartburn not related to reflux (i.e. functional heartburn), features characteristic of GERD in ME were selected by comparing the images from ERD and BE patients, in whom endoscopic diagnosis of GERD was evident and who were considered a positive control group, with the negative control group. The negative control group consisted of patients without the symptoms of GERD, not treated with PPI, having normal result of conventional endoscopy. An indication for upper gastrointestinal endoscopy in this group included exclusion of GERD or peptic ulcer disease in the course of qualifying for bariatric procedures. Patients from the negative control group were subjected to 24-hour esophageal pH-impedance monitoring with results indicating normal values. All the patients and the negative controls were subjected to endoscopy according to the same protocol. Finally, we evaluated if the abnormalities identified in ME are useful to diagnose GERD in patients with negative result of conventional endoscopy (NERD patients).

Sixty seven patients (35 males, 32 females) with clinical symptoms of GERD were enrolled into the study. Among them, there were 25 patients with BE undergoing surveillance and 42 GERD patients referred for upper gastrointestinal endoscopy for the first time. In the latter group, 3 patients were diagnosed with BE, 11 with ERD and 28 with NERD. Altogether, the study group consisted of 28 patients with BE (41.8%), 11 patients with ERD (16.4%) and 28 patients with NERD (41.8%).

The negative control group consisted of 12 patients (6 males, 6 females) without the symptoms of GERD and normal result of conventional endoscopy.

The average age was 44.8 ± 22.5 in ERD, 57.8 ± 14.4 in BE, 46.7 ± 16.3 in NERD patients and 52.2 ± 22.2 in the negative control group. Patients with BE were significantly older than those with ERD and NERD ($p < 0.05$).

2.2. Endoscopy

All the patients and controls underwent conventional endoscopy of the whole upper gastrointestinal tract and further detailed examination of the mucosa proximally and distally to Z-line using optical magnification up to x115 (Olympus GIF Q160Z). Patients received 1% lidocaine topical orolaryngeal anesthesia. Midazolam was given intravenously according to the individual needs. All endoscopies were performed by one endoscopist experienced in ME (J.W-B).

On the basis of the appearance of esophagus in conventional imaging the patients were divided into 3 groups: BE, ERD and NERD. BE was diagnosed if gastric or intestinal metaplasia was histologically proved above the gastro-esophageal junction according to the Montreal criteria [4]. Diagnosis of ERD followed Los Angeles classification [17].

The magnified images were analyzed with respect to the following lesions:

1) Microerosions - mucosal breaks invisible prior to magnification (Fig. 1b);
2) Big triangular indentations of columnar mucosa into the squamous mucosa involving more than 1/3 of radial gastric fold (Fig. 1c);
3) Serrated Z-line defined as small indentations into the squamous mucosa involving less than 1/3 of radial gastric fold, with the length of each greater or equal to its width (Fig. 1d);
4) Blood vessels visible through columnar mucosa (Fig. 1e);
5) Villiform columnar mucosa below Z-line (Fig. 1f);
6) Abnormal IPCLs within squamous mucosa – increased in number or enlarged (elongated, dilated) (Fig. 1g);
7) White points – whitish spots encircling IPCLs (Fig. 1h) or irrespective of IPCLs (Fig. 1i);

8) Obscured palisade vessels within squamous mucosa – feature present if the visibility of palisade vessels was lost, with the exception of the area up to 5 mm above the Z-line (Fig. 1g, 1 h, 1i). In that area palisade vessels were obscured in nearly all patients and in controls what was then considered physiological.

Endoscopic features were evaluated qualitatively. In the case of lesions listed as 1–4, one lesion was enough to consider the feature present. The lesions listed as 5–8 were considered positive no matter if they were present on the whole or only part of the esophageal circumference.

2.3. Interobserver agreement

During each examination still images were recorded. To evaluate the level of interobserver agreement 56 representative endoscopic images of esophageal mucosa in magnification were presented to 4 endoscopists not experienced in ME. They evaluated these pictures twice. Before the first evaluation they became acquainted with the written definition of each feature backed with a representative photo. Before the second evaluation 15 min individual tutorial was carried out by an experienced endoscopist. Agreement was evaluated for each endoscopic feature separately to select the most objective one.

2.4. Histology

Three to four biopsy specimens for the histopathologic examination were taken 1–2 cm above the Z-line. If any abnormalities were observed in the ME, biopsies were collected from the locations with the most apparent lesions but macroscopically visible erosions were omitted. If no lesions were detected in magnification, biopsies were collected from 4 quadrants of the esophageal circumference. After hematoxylin-eosin staining specimens were evaluated in light microscopy by one pathologist experienced in digestive tract pathology, who was blinded to the result of the ME. Bioptates were adequate for evaluation if both basal cell layer and at least one papilla were present in the specimen. The following features were considered characteristic of GERD in histology: papillae length exceeding 2/3 of epithelial thickness, basal cell layer hyperplasia exceeding 15% of epithelial thickness and inflammatory cells infiltration [12]. More than 10 leukocytes per high power field were regarded abnormal. Other features studied by the pathologist included: the type of cells comprising inflammatory infiltration (eosinophils, neutrophils, lymphocytes), the number of papillae per high power field, the number and height of IPCLs inside papillae and the presence of acanthosis.

The protocol of the study was approved by local Ethics Committee of the Medical University of Bialystok (approval number R-I-002/115/2012). All the patients signed informed consent before the examination.

2.5. Statistics

Statistical analyses were performed using SPSS Statistics package. The Shapiro-Wilk test was used to examine for normal distribution. Parametric *t*-test or Mann-Whitney U-tests were applied as appropriate. Results were presented as mean values $\pm$ standard deviation ($\pm$ SD). For qualitative data chi-squared or Fisher's exact test was used. For all statistical tests $p < 0.05$ was considered significant. Sensitivity, specificity, positive and negative predictive value of endoscopic and histologic features for diagnosis of GERD were calculated comparing their frequency in the positive control group (together BE and ERD) and the negative control group.

Interobserver agreement was calculated as kappa values. Values ranging 0.01-0.20 were interpreted as slight agreement, 0.21- 0.40 as fair agreement, 0.41- 0.60 as moderate agreement, 0.61-0.80 as substantial agreement and 0.81- 0.99 as almost perfect agreement.
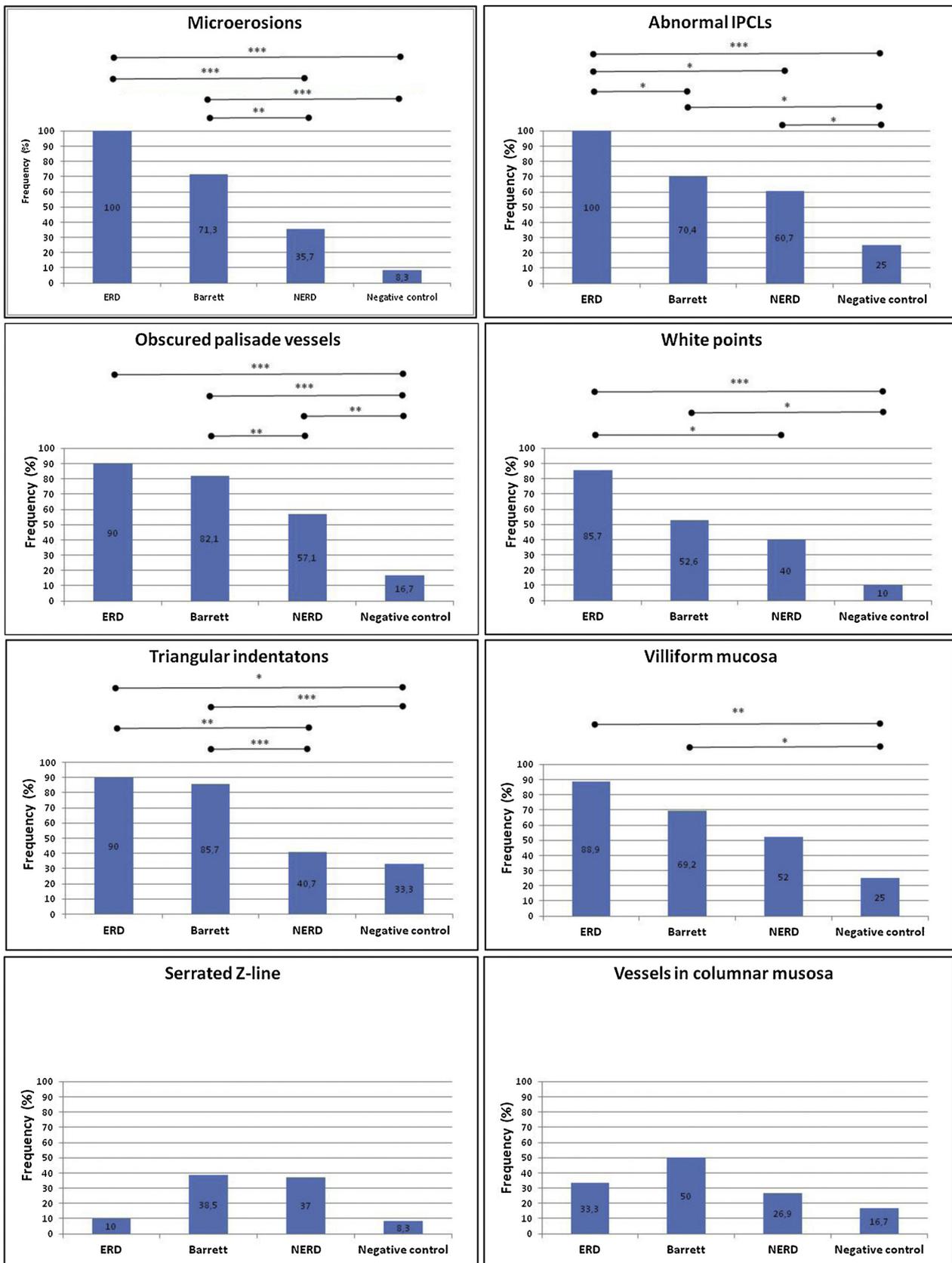
Fig. 2. Frequency of lesions identified by magnifying endoscopy in patients with ERD, BE, NERD and negative control group. The bars represent percentage of patients with the presence of particular features. Significant differences between the groups are matched as: * p < 0.05, ** p < 0.01, *** p < 0.005.

Table 1

Data concerning sensitivity, specificity, accuracy, positive and negative predictive values of endoscopic features visible in magnification for diagnosis of GERD. Calculation based on comparison between positive control group (ERD and BE patients) and negative control group (patients without GERD).

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)
Microerosions	79.5	91.7	96.9	57.9	82.4
Palisade vessels obscured	82.1	83.3	94.1	58.8	82.3
Abnormal IPCLs	76.9	75.0	90.9	50.0	76.5
White points	41.0	91.7	94.1	32.4	53.0
Triangular indentations of Z-line	84.6	66.7	89.2	57.1	80.4
Serrated Z-line	28.2	91.7	91.7	28.2	43.1
Villiform mucosa below SCJ	66.7	75.0	89.7	40.9	68.6
Vessels below SCJ	41.0	83.3	88.9	30.3	59.0

3. Results

3.1. Abnormalities visible in ME

Six features were significantly more common in both of the positive control groups (ERD and BE) than in the negative control group and therefore characteristic of GERD. They included: microerosions, obscured palisade vessels above Z-line, abnormal IPCLs, white points, triangular indentations of Z-line and villiform mucosa below Z-line. Out of these features, obscured palisade vessels and abnormal IPCLs were also significantly more common in NERD than in the negative controls (57.1% *vs* 16.7%, $p < 0.01$ and 60.7% *vs* 25.0%, $p < 0.05$, respectively). Serrated Z-line and vessels visible below the Z-line were more frequent in the GERD patients than in the negative controls, but the differences did not reach statistical significance. The frequency of all the studied features in the particular groups of patients and the statistically significant differences are shown on Fig. 2. Data concerning sensitivity and specificity, accuracy, positive and negative predictive values of endoscopic features for GERD are shown in Table 1.

We compared endoscopic lesions to histologic findings. Enlarged IPCLs reported by endoscopist correlated with the number of IPCLs inside papillae counted by the pathologist ($p < 0.05$) but not with the elongation of the papillae or height of IPCLs inside the papillae. White points correlated positively with the increased number ($p < 0.005$) and length of papillae ($p < 0.05$), together with the inflammatory cells infiltration ($p < 0.005$) and acantosis ($p < 0.005$). Moreover, the arrangement of white points was similar to the distribution of the pathological IPCLs. Therefore, our observations suggest that white points represent abnormal papillae. We did not find any histopathological abnormality that could explain the lack of transparency for the palisade vessels.

All of the evaluated endoscopic lesions appeared both in the patients with GERD and in the negative controls. The median number of the lesion types identified per patient was 5 in the ERD and BE groups, 3 in the NERD group and 1 in the negative control group. The difference was statistically significant between the negative and both of the positive control groups (negative vs. ERD and negative vs. BE $p < 0.001$) and between the negative control group and NERD ($p < 0.005$). If we assumed 2 or more types of lesions coexisting in one patient as a cut-off point for GERD the sensitivity would be 97% and specificity 75%. Fig. 3 shows the number of types of those lesions diagnosed in each particular patient. Two or more types of lesions were found in 26 out of 28 NERD patients. Sensitivity and specificity of 2 lesions cut-off point for NERD was 92.8% and 75.0%, respectively. ME was superior to conventional endoscopy because it allowed to confirm GERD not only in the BE and ERD but also in the majority of NERD patients.

3.2. Interobserver agreement

Interobserver agreement and the influence of individual tutorial differed among particular endoscopic lesions (Table 2). Initially,
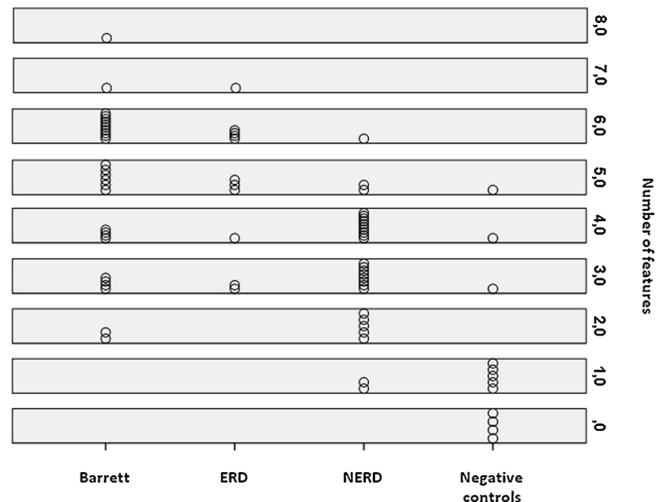

Fig. 3. Number of endoscopic features out of 8 evaluated types coexisting in individual patients from BE, ERD, NERD and negative control group. One circle = one patient.

moderate agreement (kappa values 0.41-0.60) was observed in the evaluation of serrated Z-line, microerosions and enlarged IPCLs. Agreement to the presence of the rest of the features was fair (kappa values 0.21-0.4). After tutorial, the agreement for serrated Z-line and microerosions remained moderate and the agreement for the presence of enlarged IPCLs, obscured palisade vessels and white points increased to substantial (kappa values 0.61-0.80). The agreement to the presence of big indentations and the presence of villi decreased.

3.3. Histology

Among the evaluated histologic features median ratio of papillae length to epithelial thickness was significantly higher in the ERD patients than in all other groups (ERD vs each other group $p < 0.05$; Table 3). In all of the patients from the negative control group the number of inflammatory cells per high power field did not exceed 10. In 66.6% of the ERD patients and 56.5% of the BE patients this number exceeded 10 ($p < 0.05$ in comparison to the negative control group). There were no differences between the groups considering the type of inflammatory cells infiltrating the mucosa, the basal cell layer thickness, the number of papillae per high power field and the number and extent of vessels inside the papillae. Acantosis was more common in BE (47.8%) and ERD (44.4%) compared to NERD (20.0%) or negative control (12.5%) but the difference reached statistical significance only between BE and NERD ($p < 0.05$), which were the largest groups. We found no significant differences in histology between the NERD and the negative control patients. Data concerning sensitivity, specificity, accuracy, positive and negative predictive values of histologic criteria for GERD diagnosis are shown in Table 4.

Table 2

Interobserver agreement to the presence of particular endoscopic lesions measured in kappa values. Values from 0.01 to 0.02 mean slight agreement, from 0.21 to 0.4 – fair agreement, from 0.41 to 0.60 – moderate agreement, from 0.61 to 0.80 – substantial agreement and from 0.81to 0.99 – almost perfect agreement.

Kappa	Microerosions	Obscured palisade vessels	Abnormal IPCLs	White points	Big indentations of Z-line	Serrated Z-line	Villi	Vessels below Z-line
First evaluation	0.43	0.37	0.51	0.24	0.32	0.57	0.35	0.33
Second evaluation	0.46	0.62	0.74	0.61	0.01	0.49	0.16	0.38

4. Discussion

The diagnosis of GERD in conventional endoscopy relies on the presence of typical erosions or features of BE. Only a limited number of patients with GERD present with such lesions so sensitivity of the conventional endoscopy in GERD diagnosis is low. Nonetheless in daily practise many patients with GERD symptoms are referred for diagnostic gastroscopy, therefore developing modalities to improve the yield of endoscopy in GERD evaluation are awaited. Our aim was to identify the minimal endoscopic changes characteristic of GERD visible in ME.

In the present study we examined patients with previously diagnosed BE and patients with symptoms of GERD verified by Carlsson-Dent questionnaire and positive response to PPI treatment. Montreal consensus recommends to diagnose GERD based on the symptoms [4]. The test with the PPI treatment was proved to be of comparable value to pH-metry in GERD diagnosis both in the patients with erosive and nonerosive disease [18,19]. Patients with NERD are a heterogenous population that according to the results of pH-metry can be divided into 3 groups: patients positive for abnormal acid exposure, hypersensitive esophagus and functional heartburn [20]. According to the ROME IV criteria, patients with functional heartburn should be excluded from GERD diagnosis and hypersensitivity to reflux is treated as condition on the border between GERD and functional disorders [21]. In our study, with the use of positive response to the PPI treatment as a patient selection criterion, we probably excluded the majority of individuals with functional heartburn and some patients with hypersensitive esophagus. However, taking into consideration the possibility of placebo effect of PPI, our NERD group was not homogenous. We selected endoscopic minimal lesions characteristic of GERD by comparing the negative control group to patients with ERD and patients with BE, which together constitute the positive control group. Endoscopic appearance of ERD and BE is typical, so the diagnosis may be stated without functional tests. After identification of characteristic lesions in that way, we analysed the NERD group.

We confirmed that in ME there are many alterations characteristic of GERD such as: microerosions, abnormal IPCLs, obscured palisade vessels, white points, big triangular indentations of the Z-line and villous mucosa below the Z-line. The prevalence of these lesions in the NERD group was lower than in the ERD and BE. However, comparing NERD to the negative control group we revealed significant differences in the frequency of obscured palisade vessels and abnormal IPCLs. The median number of coexisting types of pathologic lesions was 5 in the BE and ERD, 3 in the NERD and 1 in the negative control group. The presence of 2 or more lesion types in one patient allowed for GERD diagnosis with 97% sensitivity and 75% specificity. The sensitivity and specificity of 2 lesions cut-off point for the group of NERD patients with positive response to the PPI treatment was 92.8% and 75.0%, respectively. Further studies are warranted to evaluate the frequency of lesions visible in ME in patients with GERD symptoms not responding to the PPI treatment and whether the presence of hereby described lesions can distinguish between the patients suffering from functional heartburn and heartburn related to reflux.

Our results do not support the findings of Edebo et al. [9] since they did not find the differences in magnified images of esophageal mucosa between the 11 NERD patients and 10 negative controls. They evaluated triangular indentations, apical mucosal breaks (microerosions), visibility of palisade vessels, IPCLs, serrated Z-line, villiform mucosa and branching blood vessels below the Z-line using endoscope with x35 optical plus x2 electronic magnification. In the present study we used higher magnification (x115) and we analysed larger group of patients.

Lv et al. [22] examined 120 GERD patients with narrow band imaging ME. They counted the number of IPCLs in the field of view and evaluated their appearance discerning dilated, prolonged and tortuous IPCLs. Because evaluation of these features is subjective, such complex divisions seem impractical. The authors concluded that not-round pit pattern below Z-line and increased number of IPCL's were more common in GERD patients than in the negative controls, but they did not find significant differences between the three types of GERD. In our study more features were analysed. We applied a simplified classification of IPCLs – into normal and abnormal. Consequently, we found differences between BE, ERD and NERD concerning the frequency of lesions detected by ME and the number of coexisting lesion types.

ME can explain the pathophysiology of some macroscopic lesions previously described as minimal-change esophagitis such as white turbidity or redness of esophageal mucosa. In our opinion the white turbidity of esophageal mucosa associated with NERD in the study by

Table 3

Histologic features in patients with ERD, BE, NERD and negative control group.

	ERD	BE	NERD	Negative control	Significance of the differences
Median ratio of papillae length to epithelium thickness (%)	70 (± 20.0)	50 (± 23.2)	50 (± 24.1)	50 (± 8.9)	p < 0.05 ERD vs all other groups
Median ratio of basal cell layer thickness to whole epithelium thickness (%)	15 (± 9.4)	20 (± 11.1)	5 (± 12.5)	15 (± 20.3)	n.s.
Percent of patients with following number of inflammatory cells per high	33.3	43.5	56.0	100.0	p < 0.05 ERD vs NC and BE vs
power field 0–10	33.3	39.1	16.0	0.0	NC
11–20	33.3	17.4	28.0	0.0	
≥21					
Median number of papillae per high power field	2	2	1	1.5	n.s.
Percent of patients with following number of vessels inside the papillae	11.1	8.7	32.0	25.0	n.s.
0	33.3	26.1	36.0	37.5	
1–3	33.3	39.1	24.0	37.5	
4–6	22.2	26.1	8.0	0.0	
≥7					
Median extent of vessels inside the papillae (%)	50 (± 36.9)	70 (± 37.8)	50 (± 33.2)	45 (± 0.37.5)	n.s.
Frequency of acantosis (%)	44.4	47.8	20.0	12.5	p < 0.05 BE vs NERD

n.s. – not significant.

vs – versus.

Table 4

Data concerning sensitivity, specificity, accuracy, positive and negative predictive values of histologic features for diagnosis of GERD. Calculation based on comparison between positive control group (ERD and BE patients) and negative control group (patients without GERD).

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)
Thickened basal cell layer	62.5	50.0	83.3	25.0	60.0
Elongated papillae	41.2	100.0	100.0	28.6	52.4
Inflammatory infiltration > 10 cells per high power field	59.4	100.0	100.0	38.1	67.5

Kim et al. [23] may correspond to the loss of transparency of squamous epithelium to the underlying palisade vessels. In our study, abnormal IPCL's often coexisted with obscured palisade vessels but one of these features usually dominated, which may be an explanation for macroscopic color change to red [24].

We confirmed that the patients with GERD had frequently obliterated palisade vessels, which are located in the submucosa. We did not find any histologic abnormality correlating with this feature. Hypothetically, the loss of transparency of the mucosa may be due to some biochemical alterations. This phenomenon may resemble the so called endoscopic enhancement, which is a loss of transparency of columnar epithelium after spraying the surface with acetic acid – the effect of denaturation of proteins.

In the present study, during endoscopy we have often seen evidently elongated IPCLs in GERD patients. Abnormal IPCLs reported by the endoscopist did not correlate with the elongation of the papillae or height of IPCLs inside the papillae reported by the pathologist. The explanation of this discrepancy between the endoscopic view and histopathology may be explained by difficulties in correct orientation of specimens before slicing. Slices other than precisely perpendicular make evaluation of length of papillae and their vessels unreliable [25]. Abnormal IPCLs were also described in other conditions such as squamous dysplasia and carcinoma [26]. The features that allow to differentiate the abnormal IPCLs in inflammatory conditions from neoplastic lesions include regular arrangement and uniform caliber. There was no case of neoplasia in histopathologic examination of biopsies collected in the present study.

To our knowledge, this is the first study describing the presence of white points in magnified view of squamous epithelium as a feature of GERD. In fact, the white points were visible on figures shown by Lv et al. [22] but they were not named. Based on the distribution of the white points and their frequent coexistence with pin-point vessel in the center, we suppose that the white spots are abnormal papillae. Statistical analysis proved the positive correlation between the presence of the white points and the number of papillae, increased proportion of papillary length to epithelial thickness, the presence of acantosis and inflammatory infiltration in histology.

Interpretation of endoscopic image depends on the observer. In the present study, after tutorial, observers achieved substantial agreement (kappa > 0.6) in evaluating IPCLs, palisade vessels and white points on representative, still images. This level of agreement is generally perceived as quite high. For comparison, Los Angeles classification proved to be reproducible with kappa value ranging 0.49-0.65 depending on the experience of the raters [27]. The agreement in evaluation of 3 out of 6 endoscopic features characteristic of GERD in the present study was in the same or higher range than the agreement in the most commonly used and worldwide accepted endoscopic classification. Unfortunately, the Los Angeles classification can be applied only to patients with ERD. Respecting the Hetzel-Dent classification, the interobserver agreement to grade 1, which corresponds to mucosal friability and erythema was worse with kappa coefficient 0.34-0.47 [28]. In the present study 4 out of 6 GERD features presented with the same or higher agreement level. In the study on the usefulness of ME by Edebo et al. [9] the interobserver variation was acceptably low only for invisibility of palisade vessels (kappa = 0.59). In the present study, after tutorial, observers

achieved substantial agreement (kappa > 0.6) in evaluating IPCLs, palisade vessels and white points on representative, still images. Low kappa values for triangular indentations of Z-line in our study can be explained by the fact that x115 magnification is in fact too high to evaluate them. The drop of agreement to the presence of villi comparing the first and the second evaluation may be due to attempts of the tutor to show a variety of other pit patterns possibly present under the Z-line during the individual tutorial, which caused confusion in the inexperienced observers.

Another interesting issue is how the endoscopic minimal lesions depend on the PPI treatment. In the present study the esophageal mucosa was evaluated after discontinuing the PPI treatment for one week. In the previous study by Edebo et al. [9] evaluating 6 patients who proceeded endoscopy with x35 magnification before and after 4-week treatment with esomeprazole at the dose of 40 mg daily, the prevalence of triangular lesions (corresponding to big triangular indentations in our study), apical mucosal breaks (corresponding to microerosions in our study), pinpoint blood vessels in the squamous mucosa (corresponding to IPCLs in our study), and the absence of palisade blood vessels in the squamous mucosa (corresponding to obscured palisade vessels in our study), were all significantly reduced following treatment with esomeprazole ($p < 0.01$–$p < 0.001$). This finding favours the connection between the lesions in ME and GERD but probably decrease the sensitivity of ME in patients on current PPI treatment. The utility of ME during the PPI treatment has to be checked in further studies the same as the velocity of disappearance and the turnout of ME lesions. ME has the advantage over standard endoscopy in which NERD cannot be recognized even after withdrawal of PPI.

Previous studies on the usefulness of histology in the diagnosis of GERD brought conflicting results. Bowrey et al. [29] demonstrated higher frequency of severe neutrophil inflammation in complicated GERD patients, but did not show any association between the basal cell thickening or papillary zone elongation and reflux disease. In our study, patients with ERD had significantly more dense inflammatory infiltration and more frequently had elongated papillae than the negative controls but there were no differences between the groups considering the basal cell hyperplasia. Histology of squamous mucosa did not allow us to differentiate between the NERD and the negative control patients. In the study by Savarino et al. [30] histologic abnormalities such as basal cell hyperplasia, papillary elongation and dilation of intercellular spaces were useful for differentiating ERD and NERD from functional heartburn patients and healthy controls. Longer cessation of the PPI and evaluation of dilated intercellular spaces in that study might have contributed to better presentation of histology. Although at present histology is not a gold standard in GERD diagnosis, still it is complementary to other methods, especially in terms of excluding conditions such as eosinophilic esophagitis, scleroderma or carcinoma. Some hopes have been associated with the quantitative assessment of the size of intercellular spaces, which are dilated in GERD [13,29]. However there is still controversy if measuring intercellular spaces can be useful in distinguishing patients with reflux symptoms from patients with functional heartburn [31].

5. Conclusions

ME revealed lesions that may be additional endoscopic diagnostic criteria of GERD. The lesions included: microerosions, abnormal IPCLs, obscured palisade vessels, white points, big triangular indentations of the Z-line and villous mucosa below the Z-line. The presence of two or more of these diagnostic criteria in one patient indicated GERD with 97% sensitivity and 75% specificity. Substantial interobserver agreement was achieved in evaluating obscured palisade vessels, abnormal IPCLs and white points. ME was superior to conventional endoscopy because it allowed to confirm GERD not only in the BE and ERD but also in 93% of NERD patients.

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Conflict of interests

Authors declare no conflict of interest.

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