

Gastro-esophageal reflux disease and Barrett's esophagus: an overview with an histologic diagnostic approach

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Summary

The first part of this overview on non-neoplastic esophagus is focused on gastro-esophageal reflux disease (GERD) and Barrett's esophagus. In the last 20 years much has changed in histological approach to biopsies of patients with gastro-esophageal reflux disease. In particular, elementary histologic lesions have been well defined and modality of evaluation and grade are detailed, their sensitivity and specificity has been evaluated and their use has been validated by several authors. Also if there is not a clinical indication to perform biopsies in patient with GERD, the diagnosis of microscopic esophagitis, when biopsies are provided, can be performed by following simple rules for evaluation which allow pathologists to make the diagnosis with confidence. On the other hand, biopsies are required for the diagnosis of Barrett's esophagus. This diagnosis is the synthesis of endoscopic picture (which has to be provided with the proper description on extent and with adequate biopsies number) and histologic pattern. The current guidelines and expert opinions for the correct management of these diagnosis are detailed.

Key words: gastro-esophageal reflux disease (GERD), microscopic esophagitis, Barrett's esophagus, intestinal metaplasia of the cardia, histology

Introduction

In the last 20 years the approach to esophageal non-neoplastic disease has dramatically changed and improved. Various reasons can explain this new interest for esophageal pathology, from the worldwide increasing incidence of esophageal adenocarcinoma, which recognizes in gastro-esophageal reflux disease (GERD) and in Barrett's esophagus (BE) its principal carcinogenic mechanisms, to the comprehension of the pathology of conditions such eosinophilic esophagitis (EE), to the description of new rare entities such lymphocytic esophagitis (LE) which represent a challenge when approaching esophageal biopsies.


Furthermore, changes in definitions of some conditions, refinement of histologic elementary lesions and application of this knowledge in routine diagnostic practice, need to be addressed and implemented by all pathologists involved in the diagnosis of gastrointestinal diseases.

This overview on non-neoplastic esophageal disease has the main aim to furnish a practical diagnostic approach to biopsy samples to the prin-

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Conflict of interest

The Authors declare no conflict of interest.

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cipal illnesses affecting the esophagus tract, on the basis of recent published recommendations, guidelines and expert opinions.

Normal Esophageal Mucosa

The esophagus is lined by a multilayered non-keratinizing squamous epithelium. In normal individuals, the basal layer (the proliferative part of the epithelium) is limited to 2-3 layers of cells and occupies less than 15% of the total epithelial thickness. Normal papillae are shorter than 2/3 of the epithelium and inflammatory cells are sparse. Intraepithelial inflammatory cells are generally represented by T lymphocytes with a mean number of 20 in Z-lines biopsies of healthy controls and less numerous in more proximal sites ¹. Immunophenotypically normal intraepithelial lymphocytes are predominantly CD8+ suppressor, while a minority are CD4+ helper T cells ².

Sparse intraepithelial Langerhans cells and very few mast cells are also present, while B lymphocytes, NK cells and macrophages are limited to the submucosal compartment. In healthy subjects, no eosinophils or neutrophils are present within the esophageal mucosa.

Gastro-Esophageal Reflux Disease (GERD)

DEFINITION

Gastro-Esophageal Reflux Disease (GERD) is defined as damage of the squamous epithelium secondary to the pathological reflux of gastric content (comprising both gastric acidic substances and bile salts from duodenum-gastric reflux) in the esophagus, causing troublesome symptoms and/or complications.

CLINICAL PICTURE

GERD is a very common condition with a prevalence approaching 20% in western countries, while in eastern countries, the prevalence is generally lower than 10% ³. The diagnosis is related to presence of typical or atypical symptoms which adversely affect the individual's well-being. Troublesome symptoms are defined on the basis of their frequency and severity: mild symptoms occurring 2 or more days a week or moderate/severe symptoms occurring more than 1 day a week ⁴.

Typical esophageal symptoms include: heartburn (retrosternal burning sensation), regurgitation (perception of flow of refluxed gastric content in the mouth or hypopharynx) and epigastric/chest pain, sometimes indistinguishable from ischemic cardiac pain. Chronic cough, laryngitis, asthma and dental erosion are the

more frequently reported extra-esophageal symptoms ^{4,5}.

On the basis of symptoms and endoscopic appearance the following conditions can be distinguished:

- *Erosive reflux disease (ERD)*: presence of symptoms and mucosal breaks at endoscopy;
- *Non-erosive reflux disease (NERD)*: presence of symptoms in the absence of mucosal breaks at endoscopy. NERD patients are reported to be 50-60% of patients with GERD;
- *Esophageal Disorders of Gut-Brain Interaction (EDGBI, previously named functional disorders)*: these are a group of disorders with symptoms related to motility disturbances, visceral hypersensitivity, altered mucosal and immune functions, gut microbiota, and/or central nervous system processing ⁶. This category includes functional esophageal chest pain, functional heartburn and reflux hypersensitivity (esophagus sensitive to the normal reflux of acidic or non-acidic material), globus (sensation of foreign body in the throat) and functional dysphagia (sensation of abnormal bolus transit through the esophageal body) ⁷.

ENDOSCOPY PICTURE

ERD is diagnosed by endoscopy when visible breaks are seen in the esophageal mucosa near to or at the GE junction. The Los Angeles classification ⁸ is the validated and most reliable and diffuse system for grading esophageal mucosal breaks (scoring based on the number and extension). The term esophagitis, grades A to D, is used to define endoscopically detectable erosive lesions. NERD and EDGBI, by definition, do not show any endoscopic abnormalities.

BIOPSY SITE

Patients with GERD are usually diagnosed on the basis of symptoms and endoscopic assessment with/without pHmetry and impedance, in the absence of any indications for routine esophageal biopsies. This is mainly due to the fact that histology does not provide additional information for patient management. Despite this, esophageal biopsies can be performed by gastroenterologists in patients with suspected GERD complications or in patients with atypical symptoms or functional disease in the absence of endoscopic alterations. For these reasons, an accurate evaluation of histological lesions in order to provide a correct diagnosis, may be important. As histologic lesions in GERD are usually limited to the distal esophagus, sampling should include the last 2 cm above the Z line (2 biopsies at 2 cm and 2 biopsies on the esophageal side of the Z line). More proximal biopsies are less informative ⁹⁻¹⁰.

HISTOLOGIC ELEMENTARY LESIONS

The term microscopic esophagitis refers to a group of histologic lesions, observed in patients with ERD and NERD reflux disease and also in some 'functional disorders.' Histologic lesions are unspecific and can also be observed in other types of esophagites and in physiological reflux. In the last 15 years, numerous studies have focused on the definition of histologic elementary lesions, sensitivity and specificity of histology, reproducibility and validation of diagnosis and demonstration of improvement of esophageal mucosal repair after surgical or medical therapy⁹⁻²⁰.

The most informative histological lesions are the following:

- *Basal cell hyperplasia* (BCH) is defined as the thickness of the squamous epithelium basal layer $\geq 15\%$ of the total thickness. The upper limit of the basal layer is defined as the level where the nuclei of epithelial cells are separated by a distance greater than their diameter. Basal cell hyperplasia is graded as mild ($< 30\%$) or marked ($\geq 30\%$) (Fig. 1) and assessment requires well oriented samples^{11,14}.
- *Papillae elongation* (PE) is defined as an elongation of papillae $\geq 66\%$ of total epithelial thickness. The upper limit of the papilla is defined as the upper limit of the vessel along its axis. It is graded as mild ($< 75\%$) or marked ($\geq 75\%$) (Fig. 2) and assessment requires well oriented samples^{11,14}.
- *Dilated intercellular spaces* (DIS) are irregular dilatations of intercellular spaces, detectable as optically empty bubbles or ladders. DIS are more prevalent in the lower half of the epithelium and around the papillae and must be differentiated from "stretching" artefacts and from intracytoplasmic vacuoles^{11,14}. DIS can be graded as small or large (in relation to the diameter of a small lymphocyte) (Fig. 3).
- *Inflammatory cells*: intraepithelial *eosinophils* are present in about 50% of patients with GERD; intraepithelial *neutrophils* are a rare finding ($< 5\%$) in patients with NERD and their presence is usually associated with erosive disease. Both are highly specific but suffer from low sensitivity. Intraepithelial lymphocytes do not play a significant role in the diagnosis of microscopic esophagitis, nor other types of inflammatory cells (mast cells, Langerhans cells, macrophages), rarely seen in esophageal biopsies¹⁰.
- *Erosions* are characterized by the presence of necrosis with granulation tissue and/or fibrin with neutrophils; *healed erosions* show fibrosis/granulation tissue covered by thin regenerative epithelium in the absence of necrosis¹¹⁻¹⁴. These lesions are mainly seen in erosive esophagitis, with a high

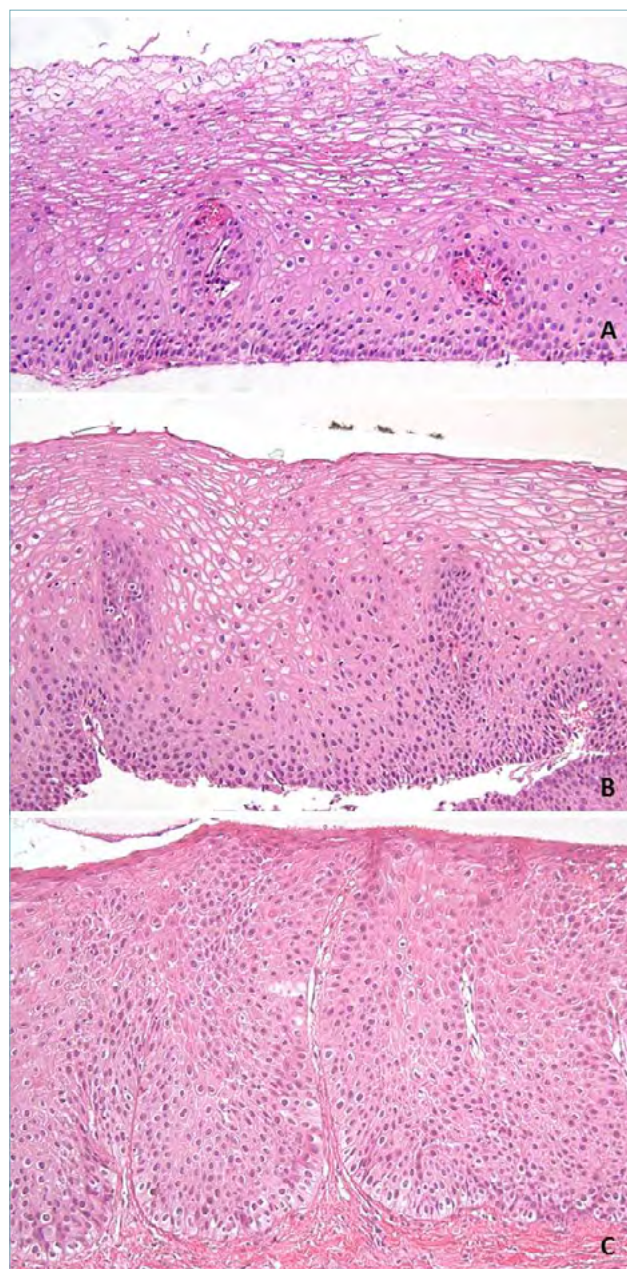


Figure 1. Elementary lesion - increasing grades of severity in basal cell hyperplasia: A) normal, epithelium basal layer thickness is less than 15% of the entire thickness; B) mild basal cell hyperplasia ranges between 15% and 30%; C) severe basal cell hyperplasia: basal cells occupy more than 30% of whole epithelial thickness. Magnification 20x. Reprinted from ref. 10 with permission from Virchows Archiv, Springer Nature.

specificity but a low sensitivity, and, together with intraepithelial neutrophils represent the most severe lesions in the spectrum of microscopic esophagitis.

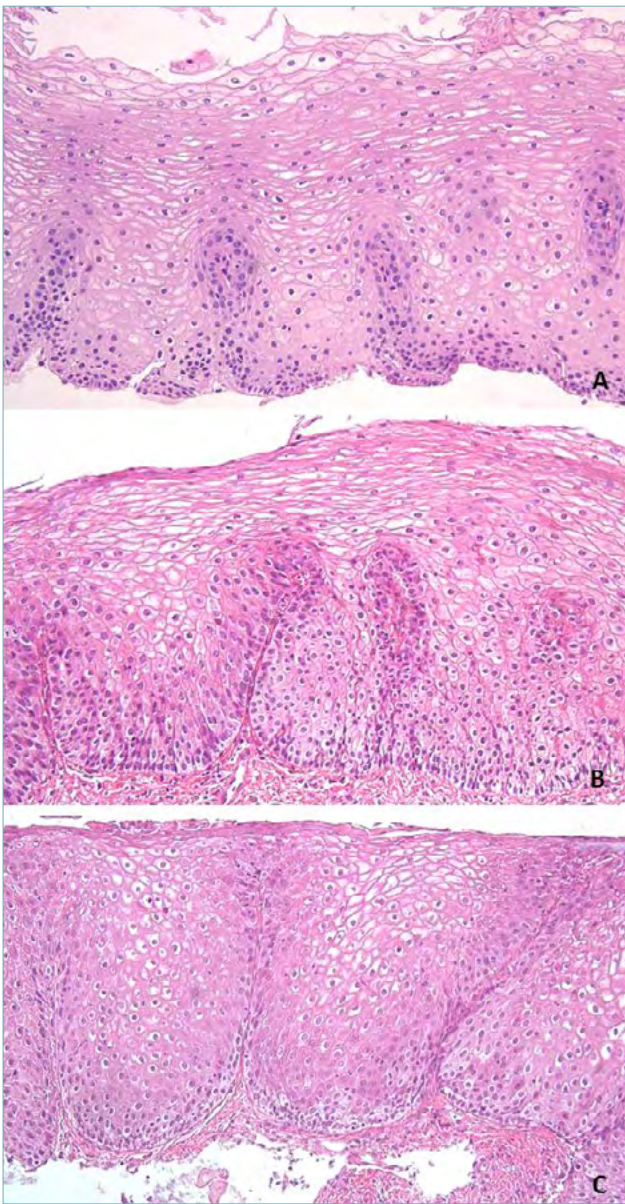


Figure 2. Elementary lesion - increasing grades of severity in papillae elongation: A) normal papillae occupy less than 2/3 of the total epithelial thickness; B) mild papillae elongation does not exceed 75% of total epithelial thickness; C) marked papillae elongation with the upper limit of papillae approaching the epithelial surface. Magnification 20x. Reprinted from ref. 10 with permission from Virchows Archiv, Springer Nature.

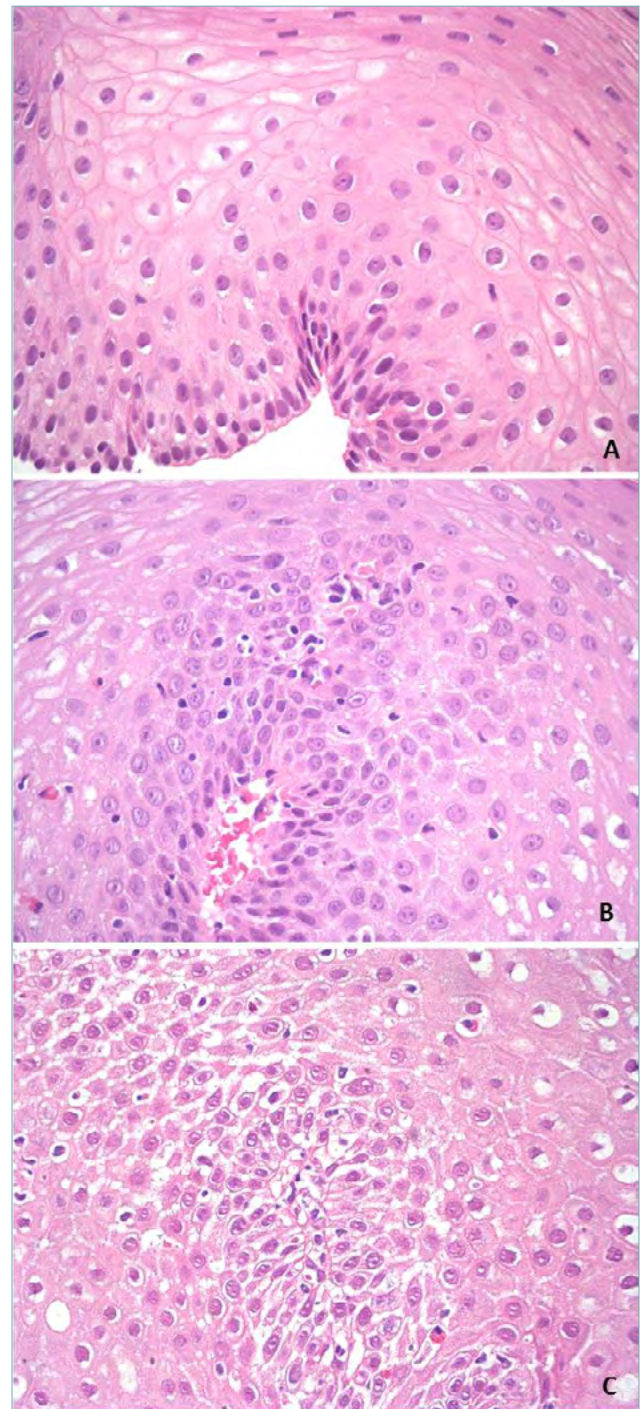


Figure 3. Elementary lesion - increasing grades of severity in dilated intercellular spaces (DIS): (A) in normal squamous epithelium, cells are sealed one with the other; (B) small, irregular DIS are shown close to a papilla; (C) large DIS with bubbles and ladders larger than the diameter of a small lymphocyte. Magnification 40x. Reprinted from ref. 10 with permission from Virchows Archiv, Springer Nature.

phagitis. Whenever they are found, microscopic esophagitis can be diagnosed regardless of the presence of other lesions.

Histologic lesions in microscopic esophagitis are irregularly distributed and may be focal. Consequently, assessment should be made in the most affected areas. A scoring system including multiple histologic lesions could help to increase both sensitivity and specificity of histologic findings¹⁰.

DIAGNOSIS

For a practical approach to esophageal biopsies, the diagnosis of microscopic esophagitis should be made when any of the following are seen: a) at least two mild histologic lesions; b) at least one severe lesion; c) any one of erosions/healed erosions/intraepithelial neutrophils^{10,21}.

Barrett's esophagus

DEFINITION

Barrett's esophagus (BE) is defined as the replacement of any portion of the esophageal normal distal squamous epithelium by metaplastic columnar epithelium, which is clearly visible endoscopically (≥ 1 cm) above the gastro-esophageal junction (GEJ). This definition requires histological confirmation on esophageal biopsies.

This definition is common to international guidelines²²⁻²⁶ as well as Italian guidelines and expert statements^{21,27}.

CLINICAL PICTURE

BE represents a complication of gastro-esophageal reflux disease, with which it shares symptoms.

The *prevalence of BE* in the population at large remains uncertain. Two studies have attempted to assess the prevalence via endoscopy screening of an unselected adult population, reporting respectively a prevalence of 1.6% in the Swedish²⁸ and of 1.3% in the Italian population²⁹. However, the limited participation rate remained a concern in both these studies, since it introduced a risk of selection bias resulting in a possible overestimate of the prevalence. On the other hand, in symptomatic patients with chronic GERD, BE prevalence is as high as 15%³⁰.

ENDOSCOPY PICTURE

The importance of measuring the length and shape of the columnar-lined segment using a standardized methodology is recognized worldwide. This aids communication between endoscopists and pathologists so improving the level of diagnostic confidence. To

obtain this goal, it is necessary to comply with recognized and standardized terminology and to precisely describe endoscopic landmarks.

TERMINOLOGY

GEJ. The term *anatomic gastro-esophageal junction* (GEJ) identifies the proximal border of the gastric folds when endoscopy is performed with minimal air-insufflation³¹. GEJ is identified exclusively by endoscopists and this term should not be used by pathologists in their diagnostic report.

SCJ or Z-line. The term *histologic squamo-columnar junction (SCJ) or Z-line* refers to the transition between esophageal stratified squamous epithelium and columnar epithelium. It can be identified both by endoscopists (due to the white color of the squamous epithelium compared to the pinkish color of columnar epithelium) and by pathologists who easily identify the histologic transition between squamous and columnar epithelium³². As a rule, GEJ and SCJ coincide in normal subjects but a dis-alignment of the SCJ up to 1 cm proximally to GEJ is also seen.

ESEM. The term *Endoscopically Suspected Esophageal Metaplasia*, according to the Montreal definition⁴ defines the presence of salmon pink mucosa in the distal esophagus at endoscopy; it describes **endoscopically** suspected columnar metaplasia related to the proximal dislocation of SCJ with respect to the GEJ.

LANDMARKS (FIG. 4)

- 1 By visualizing two *landmarks*, namely the *distal end of the palisade vessels*³³ and the *proximal end of the gastric folds*³⁴ at endoscopy, it is possible to accurately delineate the **GEJ** and identify whether there is a columnar-lined segment in the lower esophagus. The two *landmarks* should coincide at the GEJ, however different factors (peristaltic or respiratory movements, esophagitis, and degree of air insufflation) can lead to inconsistencies between these two landmarks. In a comparative study of the two methods, investigators found that the proximal extent of the gastric folds was more accurate compared to the palisade vessels (due to frequent lower position of these compared to the GEJ)³⁵.
- 2 The **diaphragmatic hiatus** is identified as an indentation of the gastric folds that is apparent during upper endoscopy with inspiration.
- 3 The **SCJ**, as mentioned above, can be recognized by endoscopists by the color changing from white to salmon pink. It is recommended that all three landmarks (or at least GEJ and SCJ), are mentioned in every endoscopic report.

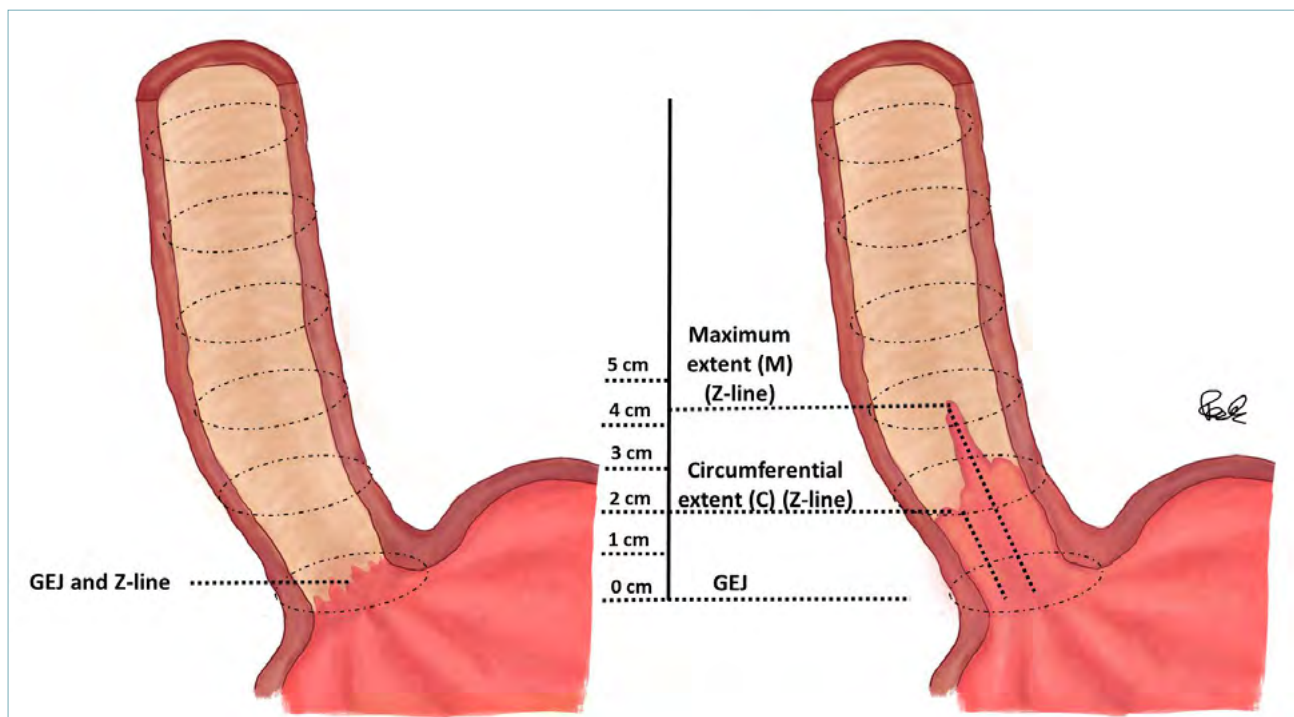


Figure 4. Landmarks (GEJ and Z-line) and the Prague C&M system for reporting ESEM length: on the left side GEJ and Z-line coincide in normal case; on the right side Z-line is proximally dislocated with respect to GEJ and a C2M4 ESEM is represented. Artwork by Federica Grillo.

REPORT ENDOSCOPICAL METHODOLOGY

The Prague C & M system for ESEM length is widely used by major academic societies such as the American Gastroenterological Association²², American College of Gastroenterology²³, the British Society of Gastroenterology²⁴ and it is also recommended by the Asia-Pacific consensus²⁵ and Australian Guidelines²⁶. The Prague C&M classification is based on validated, explicit, consensus-driven criteria³¹ and includes assessment of the circumferential (C) and maximal (M) extent of ESEM. The overall reliability coefficients for endoscopic recognition of ESEM ≥ 1 cm was 0.72. These findings have been reproduced in different patient populations^{36,37} and have also been validated in a multicenter study³⁸. A subtext in the Prague classification, records non-continuous ESEM islands (which may be found after endoscopic therapy); these last findings should also be recorded according to the Paris classification³⁹.

BIOPSY SITE

Intestinal metaplasia (IM – which in some countries is necessary for a diagnosis of BE – see paragraph below) can be patchy⁴⁰ and multiple biopsies are required in order to characterize ESEM, their number

being correlated to ESEM length⁴¹. The Seattle biopsy protocol, which entails four-quadrant random biopsies every 2 cm in addition to targeted biopsies on macroscopically visible lesions, is recommended at the time of diagnosis and at subsequent surveillance⁴²⁻⁴⁴. Unfortunately, this sampling protocol is not frequently performed in routine practice because it is lengthy and poorly tolerated by patients. Targeted biopsy samples from visible lesions should be taken before random biopsies and distal areas should be biopsied first starting 1-2 cm above the GEJ and advancing proximally. In patients with ESEM without confirmation of IM despite adequate number of biopsies, a repeat examination could be considered in 1-2 years based on a longitudinal cohort study demonstrating that around 30% of these patients can be expected to demonstrate IM on a repeat examination⁴⁵. Advances in chromoendoscopy (methylene blue, indigo carmine, and acetic acid), endoscope digital enhancements (narrow-band imaging, i-SCAN, Fujinon intelligent chromo endoscopy), and enhanced magnification have not been shown to be superior to the currently accepted practice of random four-quadrant biopsies at 2-cm intervals⁴⁶⁻⁴⁸. Biopsies of a normal or irregular z-line are not recommended. If biopsies are sampled from an irregular

Z-line, with no clear endoscopic evidence of Barrett's, they should then be sent to the pathologist as z-line biopsies and not as endoscopically suspected Barrett esophagus (ESEM) biopsy samples.

HISTOLOGIC ELEMENTARY LESIONS

Two types of columnar epithelium may replace esophageal stratified squamous epithelium: cardiac/oxynitic atrophic type and intestinal type epithelium⁴. IM in BE is most commonly of an incomplete (type II or III) subtype comprising mucous cells and goblet cells, although a complete type (type I with absorptive cells) may also be seen⁴⁹.

Finding mature oxyntic epithelium on distal esophageal biopsies is generally a sign of hiatus hernia whereas it represents gastric ectopia (inlet patches) when found in the mild-proximal esophagus.

What defines the histologic diagnosis of BE still remains a contentious issue. Indeed the type of columnar mucosa necessary for BE diagnosis varies between different countries. While the American College of Gastroenterology - ACG⁵⁰ and Australian guidelines²⁶ require IM as a necessary diagnostic criterium to diagnose BE, the British Society of Gastroenterology guidelines²⁴ and the Asia-Pacific consensus on the management of GERD²⁵ both suggest that in the context of visible columnar epithelium, IM is not a requisite, and hence gastric cardiac/oxynitic atrophic type metaplasia is also regarded as BE. Both points of view have some merit:

- a the emphasis on *IM as a defining feature of BE* is based on an increasing body of evidence which has demonstrated an increased risk of neoplastic progression for ESEM with IM compared to ESEM without IM. Among various studies, one of the largest population-based cohort investigations demonstrated a substantially higher EAC risk in subjects with columnar metaplasia with IM compared with those without IM (0.38%/year vs 0.07%/year, $p < 0.01$)⁵¹. Furthermore, a detailed genomic analysis comparing IM and non-IM epithelium in 45 patients with BE reported a higher frequency of mutations in cancer-associated genes such as CDKN2A, WWOX, c-MYC and GATA6 in IM⁵². However, other studies have not corroborated such findings⁵³⁻⁵⁴.
- b *IM is not necessary for BE definition* as sampling may impact on IM detection. Within the length of ESEM, IM has been shown to be patchy and generally found with greater frequency on the squamo-columnar junction leading edge⁵⁵. This may lead to sampling error with misclassification of ESEM with IM as ESEM without IM. The yield for IM correlates directly with the number of endoscopic

biopsies obtained. In a large retrospective study, the yield for IM was 35% if 4 biopsies were obtained, and up to 68% after 8 biopsies were performed⁵⁶. Biopsy site is also relevant for IM detection, with a 94% detection rate of IM when biopsies are performed close to the squamo-columnar junction, even if fewer samples are taken⁵⁵. Finally, recognition of IM was shown to increase cumulatively with follow-up: over 50% of patients who originally did not have IM were found to have IM at 5 years' and over 90% at 10 years' follow-up⁵⁷.

Few studies focus on *inter-observer variability in the diagnosis of BE*. In particular, one study⁵⁸, reported an inter-observer agreement of 88.3% (kappa value of 0.41) in distinguishing columnar epithelium types. An Italian study, evaluated both the inter-observer agreement in type of epithelium recognition (oxyntic/cardia versus intestinal) and more importantly on diagnostic category assignation. While agreement for diagnosis of BE had a moderate-substantial agreement rate among participants (overall $K = 0.60$ 95% CI 0.58-0.62) major problems arose when interpreting columnar epithelium in an irregular z-line³². This perfectly describes the frequent and diffuse problems faced by practicing pathologists in esophageal biopsy assessment, and therefore in BE diagnosis. In our opinion this point needs to be addressed further, as clear, reproducible and accurate BE diagnoses are the first, indispensable step for access to surveillance programs.

Ancillary techniques. There is an undeniable need for novel diagnostic approaches to the evaluation and risk stratification of patients with BE. Unfortunately, currently available ancillary techniques, including histochemical and immunohistochemical markers, have little to offer over routine H&E assessment, because they lack sufficient specificity for detection of BE and dysplasia classification⁵⁹. Some such biomarkers have been investigated and are here briefly described. *Alcian Blue-PAS* helps distinguish cardia from intestinal epithelium. Unfortunately, the specificity of Alcian blue for goblet cells is generally low, particularly with respect to distinguishing goblet cells from their morphologic mimics. Johnson and colleagues found that Alcian blue detects goblet cells with similar sensitivity (100%), but lower specificity (90%), compared with H&E, owing to false-positive staining of esophageal mucus glands and columnar non-goblet cells⁶⁰. There is insufficient evidence to justify the reflexive use of Alcian blue and/or PAS on all esophageal biopsies because goblet cells are almost always identifiable on routine H&E-stained sections⁶¹. The value of *immunohistochemical markers* in establishing a diagnosis of BE has been diffusely investigated applying numerous markers of intestinal

phenotype (CDX2, MUC2, MUC1, Villin, SOX9 and DAS1). Although these stains may be markers of an earlier phase of intestinal differentiation, there is insufficient evidence to suggest that they predict the development of IM. Furthermore, their use to distinguish between IM in BE from IM of the cardiac has been largely unsuccessful⁶²⁻⁶⁵.

DIAGNOSIS

According to the previous terminology suggested for BE diagnosis in the histology report by SIA-PEC-IAP²¹, we suggest the use of the following terminology (Fig. 5):

- Barrett's esophagus with Intestinal Metaplasia (when IM is histologically proven in an *ESEM* ≥ 1 cm at index biopsies);
- Barrett's esophagus without Intestinal Metaplasia

(when IM is not demonstrated in an *ESEM* ≥ 1 cm at index biopsies).

These two different diagnoses will lead to different management and follow-up.

No diagnosis of BE can be made on biopsies taken from *< 1 cm z-line irregularities*; the type of columnar epithelium in GEJ biopsies should be described differentiating between oxyntic/cardial epithelium suggestive of site-appropriate gastric mucosa and intestinal epithelium suggestive of intestinal metaplasia of the cardia.

Conclusions

The role of histology in diagnosing microscopic esophagitis related to GERD is still debated, however, in

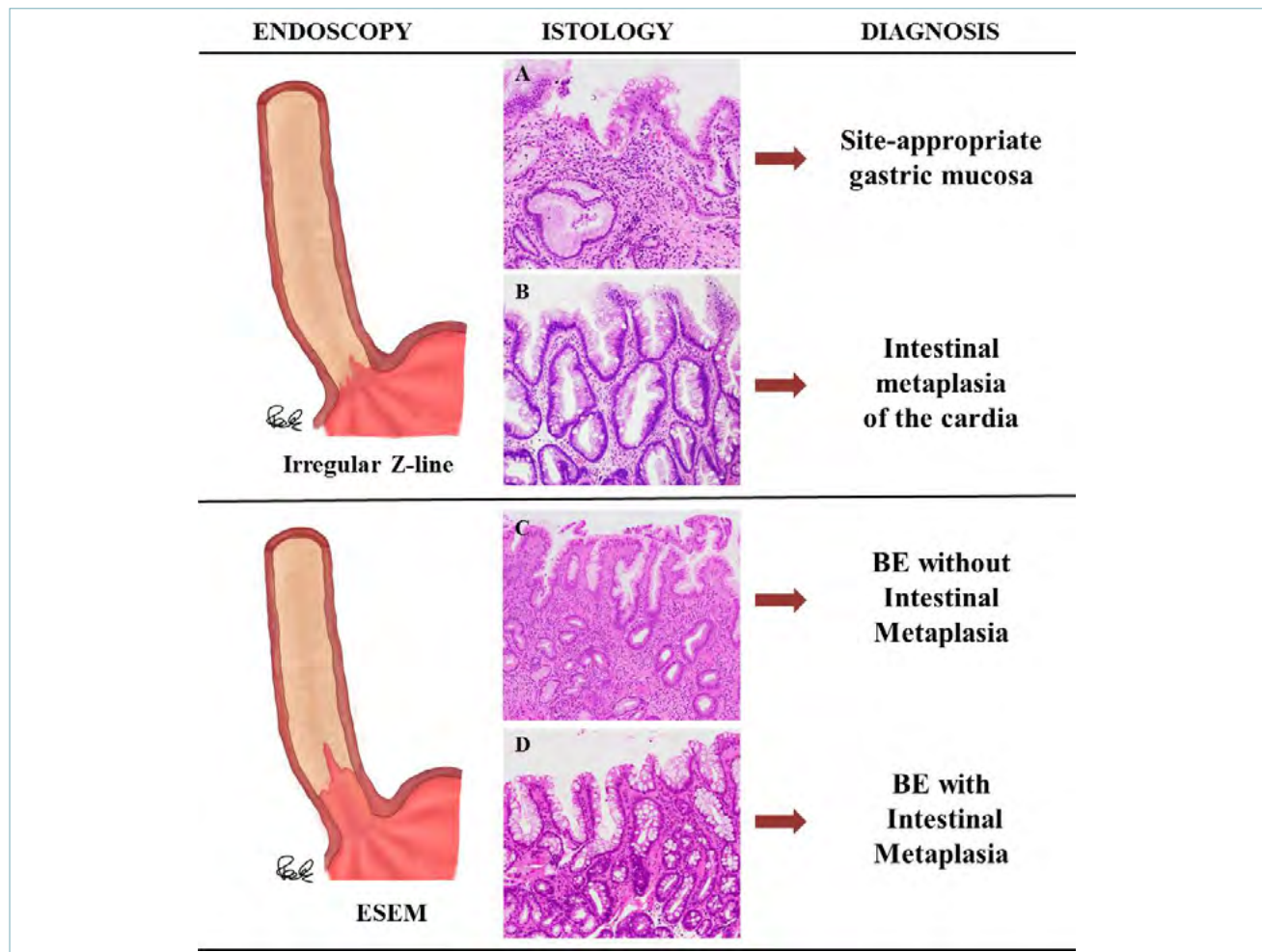


Figure 5. Pictorial representation of the diagnostic algorithm taking into account endoscopic and histologic findings to correctly interpret the columnar lined esophagus. (A-C) Columnar cardia-type epithelium; (B) Columnar intestinal-type epithelium. BE: Barrett's Esophagus. Artwork by Federica Grillo.

the last 20 years or so, histologic elementary lesions have been more precisely described and defined. Though biopsies are currently not part of the routine work up in diagnosing GERD, it is important to recognize and properly evaluate histologic elementary lesions, in order to make this diagnosis only when clear damage of the squamous mucosa is seen. The identification of true lesions will hopefully contribute in maintaining the high sensitivity and specificity that histology has demonstrated to have in this setting. On the other hand, biopsies are mandatory in diagnosing GERD-related complications, and in particular BE. The diagnosis of BE suffers from a low inter-observer reproducibility related to different reasons (ie different BE definition in different countries, availability of precise endoscopic description and sampling). This diagnosis has however a major impact both in deciding which patients need to be included in surveillance programs and in making these programs cost-effective. For these reasons it seems to be very important to precisely describe the type of columnar epithelium present in esophageal biopsies, to apply the algorithm (histology plus endoscopy) for diagnosis, and to refer to the adopted current guidelines (whichever are chosen and referenced) in order to make this diagnosis as clear as possible for the clinician.

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