Changes in the esophageal mucosa of patients with non erosive reflux disease: How far have we gone?

Christos Triantos, Nikolaos Koukias, Georgios Karamanolis, Konstantinos Thomopoulos

Abstract
The normal esophageal mucosa creates a protective epithelial barrier that constrains the acidic reflux in the esophageal lumen. Microscopic findings and functional studies indicate that this barrier might be impaired in patients with non erosive reflux disease (NERD) but not in patients with functional heartburn (FH). Whereas endoscopy and pH monitoring are the most important diagnostic tools in the diagnosis of NERD, recent studies suggest that esophageal biopsies might have a complementary role. Particularly in the differential diagnosis between NERD and FH, the application of histological severity scores showed very promising results. Further evaluation of the scores could lead to routine application of histology in specific NERD populations.

Key words: Esophageal mucosa; Non erosive reflux disease

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Core tip: The normal esophageal mucosa creates a protective epithelial barrier that might be impaired in patients with non erosive reflux disease (NERD). Whereas endoscopy and pH monitoring are the most important diagnostic tools in the diagnosis of NERD, recent studies suggest that esophageal biopsies might have a complementary role. Particularly in the differential diagnosis between NERD and functional heartburn, the application of histological severity scores showed very promising results. Further evaluation of the scores could lead to routine application of histology in specific NERD populations.

INTRODUCTION
An increased prevalence of gastroesophageal reflux disease...
ESOPHAGEAL MUCOSA IN NERD

The most extensively studied finding in the esophageal epithelium of NERD patients is the presence of dilated intercellular spaces (DIS). It has been proposed as a mechanism of impaired mucosal integrity and increased acid perception. Acid perfusion in the esophagus of healthy volunteers caused dilation of intercellular spaces in initially normal epithelium. Increased mucosal permeability due to DIS could permit the acidic fluid reach the sensitive esophageal nociceptors that terminate in the intercellular space. Moreover, an experimental study showed that not only acidic but weakly acidic solutions containing bile acids could also provoke increased DIS. It has been found that in NERD patients the mean intercellular space diameter in distal esophagus is threefold higher compared with controls. PPI treatment resolves symptoms and normalize DIS, whereas DIS were still increased in refractory heartburn patients despite double PPI dose. In parallel with DIS, an upregulation of specific desmosomal and tight junction proteins has been shown. This change could represent a mucosal reaction towards recovery of the epithelial barrier.

Hyperplasia of the basal layer of the epithelium and elongation of the papillae that are more prevalent in the mucosa of NERD patients compared to healthy controls and functional heartburn patients, are other interesting findings. It has been proposed that these findings represent a regenerative response to reflux induced mucosal damage. Comparing these markers to DIS, DIS shows higher sensitivity and specificity for the diagnosis of NERD, although it is found present in up to 30% of asymptomatic healthy subjects. Thus, the lack of specificity and sensitivity make these markers of limited use for the diagnosis of NERD.

The functional integrity of the esophagus has been assessed in vitro and in vivo. In vitro assessment is made with the use of the Ussing chamber technique which includes the placement of an esophageal mucosa specimen in an aperture that separates two solutions. The transepithelial resistance (TER) is then calculated. TER is indicative for the functional integrity of the mucosal barrier that separates the luminal from the basal side of the epithelium. When esophageal biopsies were exposed to acidic solutions the impairment in integrity as measured by TER was greater in NERD patients compared to controls, indicating a defective mucosal barrier. In vivo functional integrity of the esophagus has been evaluated with the application of multichannel esophageal impedance catheter. It has been shown that NERD patients had lower baseline esophageal impedance compared to FH patients and controls, thus supporting the hypothesis of increased mucosal permeability to ions and therefore increased sensitivity to acid.
software aided assessment of baseline impedance could add diagnostic information in the routine application of pH-impedance measurements.

Finally, an immune mediated mechanism has also been investigated in the pathogenesis of NERD. It has been suggested that reflux might stimulate proinflammatory cytokine production (e.g., interleukin 8) by the esophageal epithelium that mediates damage of the esophageal tissue\(^{(11)}\). IL-8 and IL-1\(\beta\) have been found upregulated in the esophageal mucosa of NERD patients when compared to controls\(^{(23,24)}\). Treatment with lansoprazole reduced the mucosal levels of both mRNA and protein IL-8 and IL-1\(\beta\)\(^{(25)}\). Additionally, upregulation of proteinase-activated receptor-2 (PAR-2) which has been demonstrated to induce proinflammatory and neuroinflammatory effects has also been found in NERD patients compared to controls\(^{(26)}\). In esophageal biopsies infiltration of the mucosa with inflammatory cells is more prevalent in NERD compared to FH patients and controls\(^{(6-8)}\).

**APPLICATION OF HISTOLOGICAL SCORES**

The poor diagnostic value of individual histological markers has led to the application of histological scores in the diagnosis of NERD. These scores take into account a combination of histological parameters associated with extensive acid reflux and have opened new hopeful perspectives on the role of esophageal biopsies.

Recently a large international group of pathologists reached a consensus regarding the microscopic lesions in esophageal biopsies of patients with GERD that could provide the histological diagnosis of microscopic esophagitis. Individual lesions were assessed: basal cell hyperplasia, papillary elongation, DIS, intraepithelial eosinophils, neutrophils and mononuclear cells. After that, a combined histological severity score was obtained by summing up lesion scores for each of the above parameters (Table 1)\(^{(27,28)}\). Evaluation of the score showed good correlation with patients’ reflux symptoms as well as good interobserver agreement\(^{(29)}\).

Savarino et al\(^{(7)}\) used light microscopy and applied the histological score in esophageal biopsies of pHmetry defined NERD and FH patients as well as in healthy controls (Table 2). Application of the score was able to differentiate patients with NERD from those with FH with an accuracy of 79%, a sensitivity of 74% and a specificity of 86%, whereas no difference was found in the prevalence of microscopic esophagitis between FH patients and healthy controls. Furthermore, in GERD patients refractory to PPIs application of a similar histological score was able to discriminate NERD and FH patients with high statistical significance \((P < 0.001)\)\(^{(30)}\). Biopsy sampling and application of histological scores is a relatively safe and inexpensive procedure in a disease with a massive financial impact\(^{(30)}\). However, limitations for the use of histological scores do exist mainly regarding the position where the biopsies should be taken. It has been shown that the distribution of the microscopic findings is patchy and

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**Table 1  Histological criteria for the assessment of microscopic lesions described by Yerian et al\(^{(28)}\)**

| Criterion | Definition and method of assessment (magnification) | Severity score |
|-----------|---------------------------------------------------|----------------|
| Basal cell hyperplasia | Measure basal cell layer in μm and express as a proportion of total epithelial thickness \((\times 10)\) | 0 (absent \(< 15\%\)), 1 (15%-30%), 2 (> 30%) |
| Papillary elongation | Measure papillary length in μm and express as a proportion \(\%\) of total epithelial thickness \((\times 10)\) | 0 (absent \(< 50\%\)), 1 (50%-75%), 2 (> 75%) |
| Dilated intercellular spaces | Include irregular round dilations and diffuse widening of the intercellular space \((\times 40)\) | 0 (\(\leq 5 \) small), 1 (1 \(> 5 \) small and \(\leq 6 \) large) 2 (\(\geq 6 \) large) |
| Small intercellular space = diameter \(< 1 \) lymphocyte | | 0 (1-2 cells), 2 (> 2 cells) |
| Large intercellular spaces = diameter \(\geq 1 \) lymphocyte | | 0 (0 cells in one high power field) |
| Intraepithelial eosinophils | Count cells in the most affected power field \((\times 40)\) | 1 (0-9 cells) |
| Intraepithelial neutrophils | Count cells in the most affected power field \((\times 40)\) | 1 (10-30 cells), 2 (> 30 cells) |
| Intraepithelial mononuclear cells | | 0 (absent), 1 (present) |
| Erosions | Assess as presence of at least one of the following: necrosis, granulation tissue or fibrin with neutrophils \((\times 10)\) | 0 (absent), 1 (present) |
| Healed erosions | Assess as presence of granulation tissue covered by thinned regenerative epithelium \((\times 10)\) in the absence of necrosis, fibrin, and neutrophils | 0 (absent), 1 (present) |
| Combined severity score | Sum of lesion severity scores divided by the number of lesions assessed (excludes intraepithelial mononuclear cells and neutrophils, and erosions/healed erosions) | 0-0.25 normal mucosa, 0.5-0.75 mild esophagitis, \(\geq 1\) severe esophagitis |

Biopsies were taken from the Z-line and at 2 cm above it.
Biopsies were taken from the squamous epithelium side of the squamocolumnar junction and at 2 cm above it.

Table 3  Histological score applied by Kandulski et al[31]

| Type of Lesion                                                                 | No changes | Mild changes | Moderate changes | Severe changes |
|--------------------------------------------------------------------------------|------------|--------------|------------------|---------------|
| Basal cell hyperplasia                                                        | 0          | 1            | 2                | 3             |
| Papillary elongation                                                           | 0          | 1            | 2                | 3             |
| Dilated intercellular spaces                                                   | 0          | 1            | 2                | 3             |
| Intraepithelial eosinophils                                                    | 0          | 1            | 2                | 3             |
| Sum score                                                                      | A cut-off value > 5 points was applied for discrimination between NERD and FH |

Biopsies were taken 3-5 cm above the gastro-oesophageal junction.

term follow up of GERD patients is the significantly lower score found in patients with treatment induced remission compared to treatment failures[32].

Another application of histological score could be the evaluation of the natural history of NERD patients especially these with a high severity score. It has been hypothesized that chronic inflammation and continued epithelial injury could have important role in the pathogenesis of Barrett esophagus[33,34], thus a long term study including a second upper endoscopy of patients with a high severity score could estimate a possible higher incidence of Barrett esophagus among these patients.

Furthermore, histological findings could be of value in the differential diagnosis between NERD and FH especially in specific subgroups: NERD patients with borderline findings in 24 h pH metry, patients reluctant or unable to undergo 24 h pH monitoring, patients with suspicion that catheter intolerance has significantly influenced the diagnostic value of the test.

CONCLUSION

The normal esophageal mucosa creates a protective epithelial barrier that constrains the acidic reflux in the esophageal lumen. Microscopic findings and functional studies indicate that this barrier might be impaired in patients with NERD but not in patients with FH. Whereas endoscopy and pH monitoring are the most important diagnostic tools in the diagnosis of NERD, recent studies suggest that esophageal biopsies might have a complementary role. Particularly in the differential diagnosis between NERD and FH, the application of histological severity scores showed very promising results. Further evaluation of these scores could lead to routine application of histology in specific
NERD populations.

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