DIAGNOSTIC METHODS IN EOSINOPHILIC OESOPHAGITIS: FROM ENDOSCOPY TO THE FUTURE

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ABSTRACT

Eosinophilic oesophagitis (EoE) is an increasingly prevalent disease in clinical practice. Nowadays it is the most frequent cause of dysphagia in young patients and the second leading cause of chronic oesophagitis. The gold standard technique for diagnosis and monitoring the disease is oesophagoscopy with biopsies, which is not without complications. Due to the lack of consensus on the monitoring of the disease, and the rise of dietary therapies, there has been a significant increase in the number of endoscopies per patient (up to ten). At the present time, non-invasive methods are being developed that make the management of these patients a less invasive and more sustainable strategy.

Keywords: Eosinophilic oesophagitis, endoscopy, eosinophil, activity index, chromoendoscopy, non-invasive diagnostic method.

INTRODUCTION

Eosinophilic oesophagitis (EoE) is defined as an emerging antigen-mediated immune disorder, characterised by symptoms of oesophageal dysfunction and eosinophilic inflammatory infiltrates in the oesophageal wall, despite treatment with high doses of proton pump inhibitors.[1,2] It is the most frequent cause of dysphagia in the population under 50 years old, and the second leading cause of chronic oesophagitis.[3] The estimated prevalence in occidental population is >50 cases per 100,000 inhabitants, and the peak prevalence in men aged between 35-40 years is estimated as >114 cases per 100,000 persons.[4] The physiopathology of this disease resides in a retarded allergic reaction mediated by T helper Type 2 lymphocytes[5] against alimentary antigens, especially to milk and cereals.[6,7] Most patients, especially in adult ages, experience intermittent and progressive dysphagia to solid foods, frequently accompanied by episodes of food impaction, which requires endoscopic desimpaction.[8] It is a disease with a strong genetic basis. It shows overexpression of genes such as *TSLP* gene, encoding the synthesis of thymic stromal lymphopoietin,[9] or *CAPN14* gene, encoding calpain-14 in response to elevated levels of interleukin 13.[10] This entails a high risk of the disease in first-degree relatives of patients with this disease, compared to the general population.

To date, the only accepted method for the diagnosis and follow-up of EoE is endoscopy with oesophageal biopsy (undertaken in at least five samples).[1,2] Although it is not pathognomonic, the presence of pseudo rings, longitudinal lines, or white exudates suggest the diagnosis of EoE.[2] During recent years, there has been a significant delay in the diagnosis of this disease (which is estimated in a media of 4 years), mainly due to the lack of consensus guides for its management and the great interobserver variability for the description of the endoscopic signs.[11,12] This fact contributes to the development of stenosis and therefore to the worsening of the clinical situation of patients.[13] With the objective of solving this deficiency in the diagnosis, a classification that homogenises the endoscopic diagnosis (Endoscopic Reference Score [EREFS] system)[14] has been proposed. Nevertheless, this system has not been evaluated in other centres.

The lack of consensus on the follow-up of this disease makes the patients subject to numerous endoscopic procedures, and more so if they are following dietary therapies, with food reintroduction protocols that require at least ten endoscopies per patient.[6] In young patients the situation is worsened due to the fact that they have to be anesthetised for the procedure, with innate risks for the patient, and its associated economic cost.[15] It is known that oesophageal endoscopy is not without complications, and even more so in this disease, which is associated with an increase in the number of mucosal tearing and perforations.[6,17] This is why there is an urge for the development of diagnostic tools that allow for the non-invasive management of these patients.

This review focuses on the diagnostic methods of EoE and aims to give both a critical overview of the currently available diagnostic strategies, as well as an update on developing techniques for the near future. With this goal, source studies and review articles were identified by systematically searching in three major bibliographic databases (PUBMED, EMBASE, and Scopus) for the period up to July 2014.

ENDOSCOPIC METHODS

White Light Endoscopy

A great variability has been seen in retrospective series describing the endoscopic signs for EoE, a fact that conditions a limited sensibility for its diagnosis.[18] Nevertheless, on prospective series, the presence of endoscopic signs has been demonstrated in 93% of patients with EoE.[19] Therefore, this increment on the detection of endoscopic manifestations, seen on prospective series in regard to the retrospective series, manifests the importance of a careful and protocolled inspection of the oesophagus, as well as the need of a systematic description of the findings. This last point is closely linked to the experience of the endoscopist making the diagnosis of EoE, meaning that inexperienced endoscopists find approximately 55% of the pathologic signs and experienced endoscopists find approximately 78.4%.[12] This interesting fact is mainly due to the variability found in their description. Regarding this, a fair-to-good interobserver agreement was found in the description of the oesophageal lines (k=0, 48) and pseudo rings (k=0, 56), but a lack of agreement was found on the exudates (k=0, 29) and endoscopic signs (k=0, 34).[11]

With the objective of unifying the endoscopic description of EoE, a new system has been proposed. The EREFS created a protocol for the description of the inflammatory signs (furrows, oedema, exudates) and the remodelling (stenosis and rings), punctuating them according to the severity of the manifestations.[14,20] Nevertheless, the correlation of these findings with histopathology has not been widely studied. In this regard, there is an ongoing collaborative study being carried out in Spain (The Spanish Study of Endoscopy and Eosinophils Correlation Assessment),[21] which is trying to correlate the endoscopic findings (according to the EREFS) with the inflammatory activity of the disease. Preliminary results from this study demonstrate a correlation between the inflammatory activity of EoE and the presence of inflammatory signs (furrows and exudates). However, oedema is present in spite of disease remission, a fact that suggests that this sign is indicative of remodelling more than an inflammatory sign in EoE.

Biopsy Samples

The inflammatory infiltrate present in EoE follows a patchy pattern along the squamous epithelium of the oesophagus.[22] This is of particular relevance when analysing the diagnostic rentability of the biopsy, as the size of the sample is only 0.002% of the oesophageal mucosa.[23] In this regard, it has been shown that when using a cutting point of ≥15 eosinophils/high power field (hpf), the increase in sensibility for the diagnosis of EoE is correlated with the number of biopsies obtained (sensibility of 73% for one biopsy, and of 100% for six biopsies).[24] Therefore, a greater number of biopsies means increased diagnostic yield. In fact, nowadays, the number of biopsies recommended by experts is eight (four for the proximal third and four from the distal third).[22,23] Moreover, biopsy sampling has been shown to be useful to indirectly evaluate the signs of remodelling of the oesophageal wall by detecting the loss of elasticity during the biopsy, known as the 'tug sign'.[25] On the other hand, it is interesting to know that ≥15 eosinophils/hpf is not an uncommon finding in patients with gastroesophageal reflux disease (GERD). Also, in biopsy samples, we can find some histologic signs that suggest the diagnosis of EoE (degranulated eosinophils, diffuse intraepithelial distribution of eosinophils, and eosinophilic microabscesses; Table 1).[26]

Table 1: Histologic signs of eosinophilic oesophagitis (EOE) and gastroesophageal reflux disease (GERD).

Histological sign	EoE	GERD
Degranulated eosinophils	Prominent	Rare
Eosinophilic microabscesses	Frequent	Uncommon
Diffuse intraepithelial distribution of eosinophils	Prominent	Rare (usually limited to the lower half)
Basal cell hyperplasia	Prominent (usually >50% of epithelial thickness)	Mild (usually <25% of epithelial thickness)
Keratinocyte vacuolation	Possible	Possible
Dilated intracellular spaces	Possible	Possible
Lamina propria fibrosis	Frequent	Rare
Lamina propria papillae	May reach upper one-third of the squamous epithelium	May reach upper one-third of the squamous epithelium

Modified by Ali et al.[26]

Chromoendoscopy

Due to the lack of agreement in the anatomic description of white light, the development of chromoendoscopic techniques seems obvious. Nevertheless, this idea has not been sufficiently evaluated, with only one study demonstrating how the application of indigo-carmine on the oesophageal surface with a catheter spray improves the visualisation of the typical endoscopic signs of this disease.[27] Directly opposing what happens in other, similar pathologies, are the optimal visualisation of the mucosa pattern, which are key for the diagnosis. Virtual chromoendoscopy with narrow band imaging (NBI) (Olympus®) without magnification has not been able to improve the diagnostic yield of white light endoscopy.[11] Although, it has recently been published that NBI with magnification can be useful in EoE,[28] being able to differentiate between three specific signs that are not detected with GERD is important: beige discolouration of mucosa, increased and congested intrapapillary capillary loops, and invisibility of submucosal vessels.[29] Other methods of virtual chromoendoscopy, lsuh as Fujinon intelligent colour enhancement (Fujinon®) and I-scan (Pentax®), have not been used for this pathology; I-Scan is currently under evaluation by our group.

Endoscopic Ultrasound (EUS)

The role of endoscopy for the management of EoE has barely been evaluated, and this is one of the reasons why it is not recommended as a first-line tool for the diagnosis and management of EoE[30] by the clinical guidelines of the American College of Gastroenterology. Nevertheless, it is known that tissue changes, such as epithelial hyperplasia, subepithelial fibrosis, and smooth muscle hypertrophy[31] that occur in EoE as a consequence of chronic eosinophilic eosinophilia, can be evaluated by EUS. High-resolution EUS (HR-EUS) has demonstrated its efficacy in the evaluation of the different layers of the oesophageal wall.[32] It has also been possible to verify by HR-EUS that the total wall thickness in patients with EoE is greater than in a control group (2.8 mm versus 2.1 mm; p=0.004), mainly because of a greater mucosal and submucosal layer, given that the circular muscle remains with a similar thickness between groups.[33]

This thickening of the wall has been confirmed in posterior series of patients,[34,35] even though this was without any statistically significant differences due to the small size of the cohort. Therefore, and given that it has recently been manifested that the response to treatment implies an improvement on subepithelial fibrosis,[36] EUS could allow us to objectively evaluate such correlation in isolated cases following an 8-week course of fluticasone (Flonase®),[28] although it would remain to quantify such correlation to evaluate response.

EUS does not only evaluate morphological changes of the oesophageal wall, it also evaluates functional alterations that come as a consequence of fibrotic remodelling phenomena. Using high

frequency probes, it has been found that there are significant functional changes on the longitudinal fibres of the oesophageal muscles, with a marked decrease on the amplitude and duration of their contractions in patients with EoE - another reason why EUS could also play a role in monitoring the functional response to different therapeutic strategies.[37] Anecdotally, EUS fine needle aspiration (EUS-FNA) has also allowed the description of histological findings associated with EoE, such as the existence of subcarinal lymphadenopathies as a consequence of the eosinophilic infiltration[38] or the existence of atypical cells without eosinophils in the oesophageal wall.[39]

Novel Oesophageal Imaging Methods

Confocal laser endomicroscopy is a novel technology capable of obtaining microscopic images of the gastrointestinal tract *in vivo* with the help of intravenous or topical fluorescein.[40] This is an attractive approach to EoE as it spares the patient from biopsies, meaning that it is a less invasive diagnostic technique. It has also demonstrated utility in the detection of adenocarcinoma over Barrett's oesophagus (BO),[41] and it has successfully been tested in the description of a case of EoE.[42]

A modality of reflectance confocal microscopy, denominated spectrally encoded confocal microscopy (SECM), is capable of obtaining images in a more agile way, and without the administration of contrast.[43] It has been able to demonstrate, by the visualisation of biopsies of patients with EoE, a very good correlation with the results obtained by conventional histology ($r=0.76$; $p<0.0001$) with a sensitivity and specificity of 100% for its diagnosis, taking as a reference ≥15 eosinophils/hpf.[44] Applying this technology to the clinical practice, a confocal microscopy capsule has been designed, in a size that makes it easy to swallow (7x33 mm). This device is capable of visualising, *in vivo*, the oesophageal epithelium of a swine.[45] Therefore, taking into account the good correlation between SECM and conventional histology, the development of this device seems an attractive tool as a non-invasive method to monitor EoE.

Multi-photon microscopy (MPM) is an imaging system capable of capturing fluorescence from tissues, and it has been used in vivo to visualise squamous epithelium in animals.[46] Taking advantage of the auto-fluorescence capacity of the eosinophil granule proteins,[47] it has successfully been tested for the detection of eosinophils in the biopsies of patients with EoE.[48] It has to be noted that the applicability of this technique in the follow-up of EoE is subject to the development of probes of MPM, that can be used in the clinical practice.

Optical coherence tomography (OCT) is one of the promising non-invasive *in vivo* optical imaging modalities capable of providing three-dimensional micro-structural information in real-time with micron-scale resolutions and 1-2 mm penetration depth in biological tissues,[49] which has demonstrated its capacity in the histological study of gastrointestinal tract mucosa of mice.[50] Recently, OCT has been evaluated on a murine model of EoE, detecting a thickening of the epithelium when compared with white-mice.[51]

NON-ENDOSCOPIC METHODS

Biochemical Markers

With the purpose of finding serological markers of this disease, total immunoglobulin E levels have been studied, with uneven results. That is, elevated levels on a varied percentage of cases.[52] This may be due the existence of atopic comorbidities in this particular type of patient, which does not behave as a marker of active disease.[53] Eosinophil-derived proteins, such as the eosinophil-derived neurotoxin and the major basic protein, have demonstrated their utility as markers of disease of tissue activity.[54,55] Nevertheless, the cationic protein of the eosinophil does not seem useful as a marker of EoE activity.[53,56] On the other hand, the eosinophil count in serum could behave as a marker of activity of the disease, but care should be taken during pollination seasons when interpreting results, given that this can influence results in patients sensitised to respiratory allergens.[53]

Radiology and Nuclear Medicine

It has been known for years that EoE shows alterations in the barium oesophagogram,[57] but its use is not recommended as a diagnostic tool by the consensus guidelines[2] as 50% of the cases can be normal.[58] Nevertheless, the use of a non-invasive tool for follow-up has been proposed. On this matter, a study has demonstrated a 50% decrease in the calibre of the oesophageal light in adults with EoE when compared to healthy controls. In spite of no differences being found on the maximal and minimal diameter of the oesophagus before and after treatment, a significant increase on the calibre was

seen in those patients that showed alterations on the basal epidermolysis bullosa.[59] Eosinophils granule mayor basic proteins have the capacity to join anionic heparin,[60] and its activity can be detected by SPECT imaging (using 99mTc-Heparin). This tool has been used with success to monitor the inflammatory activity of the disease by incubating the biopsies of patients with active and inactive disease after a diet.[61]

Exhaled Nitric Oxide

Fractional exhaled nitric oxide (FeNO) has been evaluated as a tool to monitor response to treatment in asthma both in children and adults, with varied results.[62,63] It has also been tested as an activity marker in EoE, with significant differences in FeNO levels pre and post-treatment (20.3 ppb [16.0-29.0 ppb] versus 17.6 ppb [11.7-27.3 ppb]; p=0.009). Nevertheless, it did not predict response to treatment to corticoids[64] and, therefore, its role as a non-invasive monitoring tool in EoE is still to be demonstrated.

Luminal Fluids and Oesophageal Cytology

The Enterotest (HDC Corporation, Pilpitas, CA, USA), is a minimally-invasive string-based technology composed of a capsule with approximately 90 cm of string, that was originally designed to detect *Helicobacter pylori* and other small intestine pathogens.[65] With this device, an oesophageal string test (OST), that extracts intraluminal oesophageal secretions and determines eosinophil-derived protein biomarkers, has been designed. This tool has been found to be efficient in the diagnosis of EoE and the monitoring of its activity.[66]

Oesophageal cytology is a method that has been scarcely studied, as it has only been developed for the diagnosis of oesophageal candidiasis. This technique could suppose a less invasive method for the evaluation of this disease as it would not be necessary to take a biopsy sample, and therefore would avoid the possible bias intrinsic in the latter, mainly due to the typical patchy infiltrates that have been previously mentioned. Another advantage of this method is that it provides an immediate diagnosis and an immediate determination of the inflammatory activity, which is particularly useful in patients with dietary therapies found in food reintroduction protocols.

Regarding this last point, our group is currently developing an oesophageal aspirate technique, whose preliminary results show a good cytology/histology correlation to assess the activity of the disease (Rodríguez-Sánchez and García Rojo. Unpublished data). The main inconvenience of this technique is how difficult it is to obtain cytology samples from the oesophagus with endoscopic devices. Therefore, it is interesting to develop devices such as cytosponge, which allows taking a cytology sample from the oesophageal wall after being swallowed like a string-capsule and freed in the stomach. This has proven to be useful in the follow-up of BO[63] and it has been successfully tested in a group of patients with EoE.[67, 68]

CONCLUSIONS

EoE is an emerging clinical entity that forces gastroenterologists to be familiarised with the constant advances in its diagnosis and management in order to correctly approach this pathology that has a high impact on the quality of life of those who suffer it. The current consensus guidelines recommend oesophagoscopy with biopsy (at least five samples) as a technique for the diagnosis and monitoring of the disease. So, the use of dietary therapies carries serial endoscopic explorations that cause high sanitary costs and risks in patients. For the diagnosis of EoE, when the accuracy of endoscopy without biopsies is assessed, we find that the main drawback is the significant interobserver variability when describing endoscopic signs. This fact makes chromoendoscopy unsuitable as a diagnostic method.

EUS could play an important role in evaluating the structural and functional impairment of the oesophagus; however, until today, it is not able to assess the inflammatory activity of the disease. Other less invasive methods, such as OST and oesophageal cytology may be attractive for non-invasive monitoring of EoE. Nevertheless, it should be tested in a larger series of patients and in different centres before adopting them as techniques of choice. Therefore, it is of prime importance to optimise and individualise the diagnostic resources focusing on the search of less invasive techniques with maximal effectiveness in the management and monitoring of the disease.

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