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World Journal of Gastroenterology (WJG) is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, Pubmed Central, Digital Object Identifier, and Directory of Open Access Journals. The 2015 edition of Journal Citation Reports® released by Thomson Reuters (ISI) cites the 2015 impact factor for WJG as 2.787 (5-year impact factor: 2.848), ranking WJG as 38 among 78 journals in gastroenterology and hepatology (quartile in category Q2).

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Pleasanton, CA 94588, USA
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Fax: +1-925-2238243
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NAME OF JOURNAL

World Journal of Gastroenterology

ISSN

ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE

October 1, 1995

FREQUENCY

Weekly

EDITORS-IN-CHIEF

Damian Garcia-Olmo, MD, PhD, Doctor, Professor, Surgeon, Department of Surgery, Universidad Autonoma de Madrid; Department of General Surgery, Fundacion Jimenez Diaz University Hospital, Madrid 28046, Spain

Stephen C Strom, PhD, Professor, Department of Laboratory Medicine, Division of Pathology, Karolinska Institutet, Stockholm 141-86, Sweden

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief, Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, CA, 9001 E. Seventh St., Long Beach, CA 90822, United States

E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
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PUBLICATION DATE

April 7, 2017

PUBLISHER

Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
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Prospective Study

Proposed criteria to differentiate heterogeneous eosinophilic gastrointestinal disorders of the esophagus, including eosinophilic esophageal myositis

Hiroki Sato, Nao Nakajima, Kazuya Takahashi, Go Hasegawa, Ken-ichi Mizuno, Satoru Hashimoto, Satoshi Ikarashi, Kazunao Hayashi, Yutaka Honda, Junji Yokoyama, Yuichi Sato, Shuji Terai

Hiroki Sato, Division of Gastroenterology and Hepatology, Saiseikai Niigata Daini Hospital, Niigata 950-1104, Japan

Go Hasegawa, Division of Cellular and Molecular Pathology, Department of Cellular Function, Niigata University Graduate School of Medical and Dental Sciences, Niigata 951-8520, Japan

Author contributions: Sato H designed the research study and wrote the paper; Nakajima N analyzed the RT-PCR data; Takahashi K analyzed the manometry data; Hasegawa G analyzed the histological data and critically revised the manuscript; Mizuno K, Hashimoto S, Ikarashi S, Hayashi K, Honda Y and Yokoyama J collected the clinical data; Sato Y and Terai S critically revised the manuscript; all authors contributed to this manuscript.

Supported by JSPS Grants-in-Aid for Scientific Research, No. 16K19332; and Takeda medical research grants.

Conflict-of-interest statement: The authors declare no competing interests.

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Correspondence to: Hiroki Sato, MD, PhD, Division of Gastroenterology and Hepatology, Niigata University Medical

Abstract

AIM
To define clinical criteria to differentiate eosinophilic gastrointestinal disorder (EoGD) in the esophagus.

METHODS
Our criteria were defined based on the analyses of the clinical presentation of eosinophilic esophagitis (EoE), subepithelial eosinophilic esophagitis (sEoE) and eosinophilic esophageal myositis (EoEM), identified by endoscopy, manometry and serum immunoglobulin E levels (s-IgE), in combination with histological and polymerase chain reaction analyses on esophageal tissue samples.

RESULTS
In five patients with EoE, endoscopy revealed longitudinal furrows and white plaques in all, and fixed rings in two. In one patient with sEoE and four with EoEM, endoscopy showed luminal compression only. Using manometry, failed peristalsis was observed in patients with EoE and sEoE with some variation, while EoEM was associated with hypercontractile or hypertensive...
peristalsis, with elevated s-1gE. Histology revealed the following eosinophils per high-power field values. EoE = 41.4 ± 7.9 in the epithelium and 2.3 ± 1.5 in the subepithelium; sEoE = 3 in the epithelium and 35 in the subepithelium (conventional biopsy); EoEM = none in the epithelium, 10.7 ± 11.7 in the subepithelium (conventional biopsy or endoscopic mucosal resection) and 46.8 ± 16.5 in the muscularis propria (peroral esophageal muscle biopsy). Presence of dilated epithelial intercellular space and downward papillae elongation were specific to EoE. Eotaxin-3, IL-5 and IL-13 were overexpressed in EoE.

CONCLUSION
Based on clinical and histological data, we identified criteria, which differentiated between EoE, sEoE and EoEM, and reflected a different pathogenesis between these esophageal EoGDs.

Key words: Eosinophilic esophagitis; Eosinophilic esophageal myositis; Peroral endoscopic myotomy; Jackhammer esophagus; Achalasia; Peroral esophageal muscle biopsy

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Core tip: Eosinophilic esophagitis has long been considered as the only eosinophilic gastrointestinal disorder (EoGD) in the esophagus. However, eosinophilic esophageal myositis, characterized by eosophageal symptoms and eosinophilic infiltration in the esophageal muscle layer, has been identified using peroral esophageal muscle biopsy. Combining clinical and histological data, we have defined clinical criteria to differentiate EoGDs in the esophagus.

INTRODUCTION
Eosinophilic esophagitis (EoE) is an allergic disorder characterized by esophageal dysfunction and histological “esophageal eosinophilia”, where eosinophilia is defined by a peak number of eosinophils per high-power field (eos/hpf) ≥ 15 in tissue samples obtained by conventional biopsy[1]. "Esophageal eosinophilia" is used to describe the histological finding of increased “epithelial” eosinophil infiltration, meaning that EoE is an epithelial eosinophilic disease. In their study of full-thickness EoE specimens, Rieder et al[2] confirmed the highest density in EoE to be in the epithelium, but with additional distribution of EoE in the submucosa, muscle layer and adventitia. Clinically, proton pump inhibitor-responsive esophageal eosinophilia (PPI-REE) and secondary causes of eosinophilia, such as parasitic infection or gastro-esophageal reflux disease, are excluded from the definition of EoE[3]. However, a subtype of EoE, with esophageal symptoms and subepithelial eosinophilia (SE) observed in the lamina propria and muscularis mucosa in esophageal samples obtained by conventional biopsy, has also been reported recently[4], and termed "subepithelial eosinophilic esophagitis (sEoE)". Using peroral esophageal muscle biopsy (POEM-b), we have also previously reported an eosinophilic infiltration in the esophageal muscle layer[5-7]. However, as this eosinophilic infiltration of the esophageal muscle layer was not identifiable using conventional biopsy, it cannot be defined as EoE. The term "eosinophilic esophageal myositis (EoEM)" has been introduced to distinguish this eosinophilic infiltration of the esophageal muscle layer from EoE and sEoE. Therefore, although EoE had previously been considered as a single eosinophilic gastrointestinal disorder (EoGD) of the esophagus, heterogeneity in the depth of eosinophil involvement has been suggested as an important clinical variable for diagnosis. However, clinical criteria for differentiating between EoE, sEoE and EoEM have not yet been established. Therefore, the aim of our study was to perform a detailed analysis of clinical data from endoscopy, manometry, laboratory tests, histological examination, and gene expression analyses to identify etiological differences between EoE, sEoE and EoEM as to establish clinical criteria to differentiate between these disorders.

MATERIALS AND METHODS
Statement of ethics
Our study was conducted as part a larger study registered with the UMIN Clinical Trials Registry (UMIN 000018685). The data were obtained from patients evaluated at the Niigata University Medical and Dental Hospital, which is a tertiary referral center in Japan. The present study was approved by our Institutional Review Board (No. 2416) and carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients prior to the start of the study.

Patients
Patients with symptomatic esophageal eosinophilia within any layer of the esophagus (epithelium, subepithelium, from the lamina propria to the submucosa, or muscularis propria) were recruited. A PPI trial was first performed for all patients and, subsequently, patients with PPI-REE were excluded from the study. The diagnosis of EoE was based on the American College of Gastroenterology (ACG) clinical guideline
### Summary of a case series of eosinophilic gastrointestinal disorders in the esophagus (n = 10)

<table>
<thead>
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<th>Diagnosis</th>
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<td>Case 10</td>
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- **EoE (Eosinophilic esophagitis)**: Symptoms were classified as **EoE**, **sEoE** (Subepithelial eosinophilic esophagitis), or **EoEM** (Eosinophilic esophageal myositis); **sEoE** was defined as the presence of eos/hpf in the subepithelium, **EoEM** was defined as the presence of eos/hpf in the muscularis propria. **EoE** was diagnosed by conventional endoscopy in 5 patients, and a subtype of **sEoE** was diagnosed in 1 patient. Patients with no eosinophilia by conventional biopsy and with a Nutcracker esophagus (NE) or Jackhammer esophagus (JE) identified on manometry were classified as **sEoE**.

- **Histological findings**: Longitudinal furrows, white plaques, fixed rings, and compression of the lumen in the esophagus were assessed. Longitudinal furrows, white plaques, and fixed rings have been previously reported as typical endoscopic findings of **EoE** and **EoEM**, with luminal compression being the only previously reported endoscopic finding of **EoEM**.

- **Manometry** was also performed in all patients using high-resolution manometry (HRM; Star Medical Co., Pte., Ltd., Tokyo, Japan), with patients in the supine position, performing 10 consecutive swallows of 5-mL of water. HRM results were evaluated using the Chicago classification criteria, version 3.0. A Jackhammer esophagus (JE) was defined by hypercontractile peristalsis, with a distal contractile integral (DCI) ≥ 8000 mmHg/s-cm. Failed peristalsis was diagnosed by a DCI < 100 mmHg/s-cm. It is important to note that the diagnosis of a Jackhammer esophagus (NE) has been eliminated from version 3.0 of the Chicago classification criteria as the significance of using a DCI of 5000 to 8000 mmHg/s-cm to specifically differentiate NE was questioned. However, we maintained NE as a possible diagnosis, based on the Chicago classification criteria published in 2011 as patients with NE in our study had symptomatic esophageal eosinophilia.

- **Histopathology**: Six conventional esophageal mucosal biopsies were performed in each case to increase the detection rate of mucosal eosinophilia. Large biopsy forceps (Radial Jaw 4 Biopsy Forceps, Boston Scientific, Massachusetts, US) were used to obtain a sufficient amount of epithelium with subepithelium.

- **Cases 7 through 10** had no visible eosinophils on conventional biopsy, but a NE/JE was observed by HRM. POEM was determined as the best therapeutic option to resolve the hypertensive/hypercontractile peristalsis, and muscle specimens were obtained by POEM-b.

---

1. Eosinophilic esophagitis: defined as ≥15 eosinophils/high-power field (eos/hpf) in any layer of the epithelium, subepithelium, or muscularis propria; **EoE**: Eosinophilic esophagitis, **sEoE**: Subepithelial eosinophilic esophagitis, **EoEM**: Eosinophilic esophageal myositis; **sEoE**: Eosinophilia in subepithelium, separately. **EoE**: Eosinophilia in the esophageal muscle layer by peroral esophageal muscle biopsy; **Symp**: Symptoms; **C**: Chest pain, **D**: Dysphagia, **F**: Food impaction; **Endoscopic findings**: Presence/absence (+/-, respectively) of longitudinal furrows/white plaques/fixed rings/luminal compression; **Manometry findings**: FP: Failed peristalsis; JE: Jackhammer esophagus; NE: Nutcracker esophagus; **Histological findings of the esophagus**: Presence/absence (+/-, respectively) of dilated intercellular space/downward papillae elongation/basal cell layer destruction. EMR: Endoscopic mucosal resection; IgE: Immunoglobulin E.
cases 9 and 10, although JE was visible, no eosinophils were identified in any of the six esophageal mucosal biopsies obtained, and a mucosal entry site for POEM/POEM-b was created using cap-fitted endoscopic mucosal resection (cEMR) to allow the full-layer of the mucosa, along with the submucosa, for analysis (Figure 1). Therefore, our histological analysis included mucosal specimens obtained by conventional biopsies for cases 1-10 and by cEMR for cases 9-10, and muscle specimens obtained by POEM-b for cases 7-10.

The maximum number of eosinophils (eos/hpf) was counted separately in the epithelium, subepithelium and muscle layer for each of the 10 cases. The mucosal histology was also assessed to identify: dilated intercellular spaces, downward papillae elongation and basal cell layer destruction. Dilated intercellular spaces and downward papillae elongation have previously been reported in cases of EoE[13,14]. Upward papillae elongation, which can often occur along with the presence of balloon cells in cases of reflex esophagitis and is considered a non-specific histological finding, was excluded from our analysis[15].

POEM-b/cEMR specimens obtained from 5 patients with achalasia (4 males; mean age 44.2 ± 9.6 years) were used as controls for eosinophil counts in our histological assessments and mRNA expression analyses (see below).

Real-time quantitative reverse transcription polymerase chain reaction in esophageal mucosal and muscle layer samples

In a previous study of EoE, a genome-wide association study was used to identify the significant locus at 2p23 susceptible of encoding Calpain14 (CAPN14) and chr5q22, which mapped to a single LD block encompassing the thymic stromal lymphopoietin (TSLP) and WDR36 genes[16,17]. CAPN14 is specifically induced in the esophageal epithelium after IL-13 treatment and leads to increasingly dilated intracellular spaces in the epithelium[18]. CAPN14 also disrupts the expression of desmoglein-1 (DSG1: barrier molecule), which triggers the entry of antigens into the esophageal epithelium. TSLP is a protein of the cytokine family and is known to promote allergic inflammation by activating dendritic cells, inducing Th2 cell responses, supporting immunoglobulin E (IgE) production, and increasing the population of phenotypically and functionally distinct basophils[19]. A set of candidate genes for eosinophil chemotaxis, including eotaxin-3 and DSG1 has also been identified by transcriptome analysis[20]. C-C chemokine receptor type-3 (CCR3), which is expressed on the surface of eosinophils, mast cells and basophils, is the chemokine receptor for eotaxin[21-23], with an elevated expression of eotaxin-3 having been reported in EoE[24]. Moreover, Th2 cells are thought to be central regulators of the hallmark features of eosinophilic diseases via their influence over Th2 cytokines, such as IL-5 and IL-13[25-28].

Based on the above, real-time quantitative reverse transcription polymerase chain reaction (real-time qRT-PCR) analyses were performed on the samples obtained by conventional biopsy, cEMR and POEM-b. Total RNA was extracted using Trizol (Invitrogen, California, CA, United States), according to the standard protocol. Thereafter, cDNA was amplified using the ABI 7700 sequence-detector system (Applied Biosystems, Foster City), with a set of primers and probes corresponding to CAPN14, TSLP, Eotaxin-3, DSG1, CCR3, IL-5, IL13, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The levels of mRNA expression were normalized to a housekeeping gene, such as GAPDH: CAPN14/GAPDH, TSLP/GAPDH, and Eotaxin-3/GAPDH. Finally, the ratio of the mRNA expression relative to mRNA expression in the control tissues was calculated, with the median value of control converted to 1.

Statistical analysis

Relevant demographic patient variables and histological findings (continuous variables) were expressed by their mean ± SD values. Levels of mRNA were expressed as the mean of EoE and EoEM, respectively, relative to the control. Levels of mRNA within the range of the control samples were deemed to be within normal limits. Abnormal increases in mRNA expression of CAPN14, TSLP, Eotaxin-3, CCR3, IL-5, and IL-13 were defined by a relative ratio exceeding the maximum value of the control samples, while a decrease in the expression of DSG1 was defined by a relative ratio lower than the minimum value of the control.

RESULTS

Our analyses included the data from 10 patients who underwent assessment for esophageal EoGDs over our study period, from July 2014 through September 2016. Among these 10 patients, 5 were diagnosed with EoE, 1 with sEoE and 4 with EoEM (Table 1 and Figure 1).

**Differences in endoscopy and manometry findings, as well as serum IgE levels, between patients with EoE, sEoE and EoEM**

Longitudinal furrows and white plaques were identified in all patients with EoE (cases 1-5), with a fixed ring visible in 2 of these 5 cases (Figure 2A). For patients with sEoE and EoEM (cases 5-10), only a luminal compression was observed, with no visible evidence of longitudinal furrows, white plaques or fixed rings (Figure 3A and Figure 4A).

HRM results in patients with EoE (cases 1-5) were variable, but with failed peristalsis observed in 4 of these 5 cases (Figure 2A, insert), with findings being within normal limits for the remaining case. The patient with sEoE (case 6) presented with failed peristalsis (Figure 3A, insert) and elevated s-IgE level (678.0
IU/mL, normal range \(\leq 173\) IU/mL). It is important to note that levels of s-IgE did vary, overall, among cases of EoE. Patients with EoEM (cases 7-10) demonstrated either hypercontractile or hypertensive contractions in the esophagus (JE: 3; Figure 4A, insert; NE: 1), with elevated s-IgE levels (324.8 ± 145.9 IU/mL).

**Histological differentiation between EoE, sEoE and EoEM**

The eos/hpf ratio values were as follows: EoE (cases 1-5), 41.4 ± 7.9 in the epithelium and 2.3 ± 1.5 in the subepithelium, identified by conventional biopsy, noting that the data for the subepithelium in case 4 were excluded because the subepithelial specimens were insufficient; sEoE (case 6), 35 in the subepithelium and 3 in the epithelium, identified by conventional biopsy; and EoEM (cases 7-10), no visible eosinophils in the esophageal epithelium and 10.7 ± 11.7 in the subepithelium (cases 7, 9 and 10), noting that the data in the subepithelium for case 8 were excluded because the subepithelium specimen obtained using conventional biopsy was insufficient. An eos/hpf ratio of 46.8 ± 16.5 was identified in tissue samples obtained from the esophageal muscle layer by POEM-b (cases 7-10). Tissue samples from control subjects were essentially devoid of eosinophils in the epithelium and muscle layer, with a few eosinophils visible in the subepithelium (4.0 ± 2.5).

Dilated intercellular spaces and downward papillae elongation were identified in the mucosal samples from all EoE patients (cases 1-5; Figure 2B). Basal cell layer destruction was visible in the case of sEoE (Figure 3B) and in all EoEM cases (cases 7-10; Figure 4B). Dilated intercellular spaces and downward papillae elongation were not visible in any cases of sEoE and EoEM.

**The characteristic mRNA expression pattern of EoE was not observed in EoEM**

The esophageal mucosal biopsy samples from EoE cases (cases 1, 3, 4 and 5), in addition to the cEMR samples from EoEM cases (cases 9 and 10, both of which included rich subepithelium tissue), were sent for
mRNA expression analyses. EoE was associated with the following fold-increase in level of expression: CAPN14, 9.9-fold; eotaxin-3, 529.2-fold; CCR3, 16.3-fold; IL-5, 160.9-fold; and IL-13, 131.0-fold. No increase in the expression of TSLP was identified in these cases (2.0-fold higher values compared to the control), while there was a decrease in the expression of DSG1 (0.69-fold).

In contrast, in EoEM, the expression levels of CAPN14, TSLP, eotaxin-3, CCR3, IL-5, and IL-13 were equal to those in controls (1.07, 2.0, 0.96, 0.79, 4.93, and 0.00-fold increases, respectively), and the expression of DSG1 was highly preserved (DSG1: 11.0-fold) (Figure 5A).

Tissue samples of the esophageal muscle-layer obtained by POEM-b in patients with EoEM were also analyzed for mRNA expression, with the following increases noted: eotaxin-3, 6.44-fold; and CCR3, 18.7-fold. Levels of TSLP, IL-5 and IL-13 (2.79, 0.00, and 0.25-fold, respectively) were within control values (Figure 5B).

**DISCUSSION**

In this study, we performed histological and gene expression analyses on a case series of EoEM, and compared those with cases of EoE and sEoE. Eosinophilic gastroenteritis (EoGE) is an EoGD characterized by eosinophilia in the stomach, small intestine or large colon, and is sometimes complicated with EoE. Heterogeneity in the depth of eosinophil involvement of the different layers of the gastrointestinal tract, including the mucosal, muscle and serous layers, has been reported in patients with EoGE[29]. In the esophagus, this heterogeneity in the depth of involvement, however, had not previously been characterized due to the difficulty in obtaining tissues samples with sufficient subepithelium, together with epithelium using conventional biopsy, due to the thickness of the stratified squamous epithelium. Furthermore, histological analyses of the esophageal muscle layer are technically difficult and invasive. In contrast, muscle layer and serous-type of EoGE, show ascites that allow for diagnosis by computed tomography and ascites puncture. To our knowledge, our study is the first to demonstrate heterogeneity in the depth of eosinophil involvement in the esophagus using a combination of conventional mucosal biopsy, cEMR, and POEM-b.

In all 5 cases of EoE, longitudinal furrows and white plaques were visible, with fixed rings observable in 2 of these 5 cases. These endoscopic findings are characteristic of EoE, although they are not
highly sensitive for diagnosing the disease\textsuperscript{[9]}. These distinctive endoscopic features of EoE are reflected as dilated epithelial intercellular spaces and/or downward papillae elongation histologically. In EoE, epithelial inflammation and subsequent fibrosis lead to peristalsis disturbances\textsuperscript{[30]}. Our HRM results in fact did identify failed peristalsis as the main finding in EoE. However, there was variability in this finding with one patient identified in whom peristalsis was deemed to be within normal limits. In patients with EoE, an allergic response to the allergen stimulated esophageal epithelial cells to produce eotaxin-3, which recruits eosinophils via the CCR3 receptor. In these patients, Th2 cytokines as IL-5 and IL-13 are also overexpressed and induce the loss of barrier integrity in epithelial cells. This process is mediated, in part, by a reduction in the expression of DSG1 and an increase in the expression of epithelial CAPN14, which leads to increasingly dilated intercellular spaces. The results of our mRNA analysis correspond to a previously reported hypothesis on this matter\textsuperscript{[26]}.

In our one case of sEoE (case 6), conventional endoscopy did not reveal any of the characteristic findings of EoE, including longitudinal furrows, white plaques, and fixed rings. In this case, elevated levels of s-IgE and failed peristalsis, as confirmed by HRM, suggested that other esophageal EoGDs could be involved and that conventional biopsy, targeting SE, could be used to diagnose sEoE. Epithelial histology did not reveal any dilation of the intercellular spaces or downward papillae elongation in this case. Luminal compression observed on endoscopy was likely caused by subepithelial inflammation secondary to eosinophil infiltration. Subepithelial inflammation may also trigger the destruction of the basal cell layer and lead to upward papillae elongation, but not a downward papillae elongation. Heterogeneity in endoscopic and histological findings has previously been reported in cases of EoE\textsuperscript{[11]}. In fact, cases with “non-EoE-like endoscopic and histological findings” are more likely to represent sEoE than EoE. A lower degree of epithelial eosinophilia, but with a similar clinical course to EoE has also been reported\textsuperscript{[30]}. In our case series, the pathological mechanism of sEoE in case 6 was suspected to be somewhat different from that of EoE based on the endoscopic and histological findings.

In cases of EoEM, a luminal compression was only identified in cases of sEoE, in contrast to the findings...
Patients with esophageal symptoms receive endoscopy. Other causes of esophageal eosinophilia should be excluded.

![Diagram of diagnosis criteria for eosinophilic gastrointestinal disorders in the esophagus.](image)

Sato H et al. Heterogeneous eosinophilic esophagitis

Luminal compression on endoscopy and failed peristalsis, as well as JE or NE on HRM, are not specific findings for sEoE or EoEM. Moreover, a past or present history of other allergy disorders can result in elevation of s-IgE and, therefore, a comprehensive clinical decision-making process is needed in such cases. Based on the premise outlined above, histological assessment by conventional biopsy is necessary to assess the full esophageal mucosal layer in patients with suspected EoGDs. Although we used large biopsy forceps for conventional biopsies in our study to obtain a sufficient volume of subepithelium tissue together with the epithelium, in some cases sufficient subepithelium still could not be obtained (cases 4 and 8). As well, several biopsies should be performed due to the patchy distribution of eosinophils in cases of EoE. Re-endoscopy with re-biopsy should also be considered if only a few epithelial eosinophils are identified in an insufficient volume of subepithelium. Diagnostic cEMR may be somewhat invasive for obtaining sufficient subepithelial tissue, and therefore, it was only performed in combination with POEM/POEM-b in our study. Endoscopic ultrasound-guided fine needle aspiration may be a good option for cases in which sEoE and EoEM are suspected.

There are several limitations in our study, which need to be acknowledged. Foremost, other disorders such as reflux esophagitis and achalasia are associated with low-grade SE, as shown in the tissue samples from patients in our control group. Therefore, a reliable cut-off number of eosinophils for the diagnosis of sEoE will need to be determined in future studies. mRNA analyses for cases of symptomatic achalasia were used as a control for two reasons. the first, tissue samples are obtained using the same POEM-b method. Second, tissue samples in achalasia do not show eosinophilia in the esophageal muscle layer. Non-symptomatic
individuals without any known esophageal disorders would provide a more appropriate control, although this would pose a difficult ethical problem. This was a small-size pilot study and further studies, including larger sample sizes, are needed to confirm our findings. In fact, we are continuing to collect data using our procedure outlined in Figure 6 with the aim of supplementing our case series in future reports. Future research should also specifically aim to include a larger number of patients with sEoE patients.

In conclusion, we propose clinical criteria for differentiating EoE, sEoE and EoEM, taking into account the histological heterogeneity in the depth of eosinophil involvement was observed among these disorders. Our findings predict a difference in the pathogenesis of these disorders, and further research will be required to fully elucidate these differences.

COMMENTS

Background
Eosinophilic esophagitis (EoE) is an allergy disorder, defined by a histologically severe eosinophil infiltration in the esophageal epithelium. EoE has long been considered to be the only eosinophilic gastrointestinal disorder (EoGD) of the esophagus.

Research frontiers
Peroral endoscopic myotomy (POEM) was developed to provide a less invasive technique to perform transoral esophageal muscle layer biopsy (peroral esophageal muscle biopsy: POEM-b). Using POEM-b, a new disorder with an eosinophilic infiltration in the esophageal muscle layer was detected, and a new name “Eosinophilic esophageal myositis: EoEM” was given.

Innovations and breakthroughs
This is the first study to have addressed the clinical differentiation of EoGDs of the esophagus. Pathogenesis of EoEM was also analyzed by real-time qRT-PCR analyses of the esophageal samples.

Applications
EoEM need to be differentiated in cases of symptomatic esophageal motility disorders.

Terminology
EoEM is defined as esophageal symptoms and histologically severe eosinophil infiltration in esophageal muscle layer. EoEM has no epithelial eosinophilia as EoE.

Peer-review
The authors presented a study which determined the criteria to differentiate heterogeneous eosinophilic esophagus. The study reflected a different pathogenesis between EoE, sEoE, and EoEM. The study is interesting.

REFERENCES

Sato H et al. Heterogeneous eosinophilic esophagitis


P- Reviewer: Garcia-Olmo D, Jurcic P, Yu CH  S- Editor: Yu J  L- Editor: A  E- Editor: Zhang FF