

Immunohistochemical differentiation of eosinophilic esophageal myositis from eosinophilic esophagitis

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Abstract

Background and Aim:

Eosinophilic esophagitis (EoE) is a Th2-mediated allergic disease of the esophageal epithelium, associated with antigen. We previously reported a case series for eosinophilic esophageal myositis (EoEM)—a novel eosinophilic gastrointestinal disorder (EoGID) defined as eosinophilic infiltration localized in the esophageal muscle layer—and diagnosed it by peroral endoscopic muscle biopsy. Here, we investigated the immunopathology of EoEM to differentiate it from EoE.

Methods:

Histological analysis was performed for three cases of EoEM and EoE, respectively. The results were compared to those of two control samples (non-EoGID full-layer esophagus). Using immunofluorescence, we analyzed the expression of the chemokine receptor CCR3 and its ligands eotaxin-1 and -3 to investigate the eosinophilic reaction. Additionally, we determined the expression patterns of desmoglein-1 in the esophageal epithelium, which shows dysregulated expression in EoE.

Results:

Eosinophil infiltration was observed in the muscle layer (maximum number, 30, 36, 73/high-power field [HPF]) and the epithelium (50, 44, 40/HPF) for EoEM and EoE, respectively. In EoE esophageal epithelium, the number of eotaxin-3-positive epithelial cells was significantly increased together with CCR3-positive infiltrating cells. However, in EoEM, the numbers of eotaxin-1- and -3-positive myocytes and vascular endothelial cells were increased in the esophageal muscle layer. A significant loss of desmoglein-1 expression was only observed in EoE, not in EoEM.

Conclusions:

Eotaxin-1 and -3 expression on the smooth muscle and vessels plays a role in the pathogenesis of EoEM, while EoE shows an epithelial eotaxin-3-dominant immunoreaction. Thus, the EoEM immunological pattern displays clear differences from that of EoE.

KEYWORDS:

Eosinophilic esophagitis; eotaxin-3; eotaxin-1; CCR3; Desmoglein-1

INTRODUCTION

Eosinophilic gastrointestinal disorders (EoGIDs) are chronic inflammatory diseases that are characterized by allergic gastrointestinal eosinophilia. EoGIDs are broadly categorized into eosinophilic esophagitis (EoE) and eosinophilic gastroenteritis (EoGE). EoE is defined as esophagus-localized eosinophilia and as excluding proton pump inhibitor-responsive esophageal eosinophilia (PPI-REE) before the definitive diagnosis.¹ Because esophageal epithelial eosinophilia destroys the normal epithelial structure, conventional endoscopy can be used to diagnose it by its eosinophil-related endoscopic features, such as a loss of vascularity, white plaques, longitudinal furrows, and fixed rings.² Therefore, EoE can be diagnosed by mucosal biopsy through conventional endoscopy. The number of EoE cases is rapidly increasing globally, particularly in western countries.

On the other hand, EoGE is diagnosed by marked eosinophilia in the stomach—often also in the esophagus, small intestine, or colon—and by ruling out other secondary eosinophilias.³ Therefore, in contrast to the esophageal localized pathology of EoE, EoGE is considered to be associated with systemic pathology. Further heterogeneity in the extent of involvement of the different layers of the intestine was reported in EoGE.⁴ This implies that EoGE can also be muscle layer type or subserosal type not involving mucosa.³ However, the detailed mechanisms of EoE and EoGE are still unknown.

Eosinophilic esophageal myositis (EoEM) was reported as a new type of gastrointestinal eosinophilia,^{5, 6} defined by eosinophil infiltration localized in the esophageal muscle layer, but never seen in the mucosa. Patients with EoEM show an almost normal esophagus on conventional endoscopy, and no characteristic features in mucosal histopathology. Therefore, EoEM cannot be diagnosed by mucosal biopsy.

Patients with EoEM may be categorized as having Jackhammer esophagus or Nutcracker esophagus on high-resolution manometry (HRM) using Chicago classification criteria.⁷⁻⁹ However, these patients may long be suffering from undiagnosed dysphagia and chest pain. This is because motility diagnosis (Chicago classification) does not have provisions for pathological observations and customized therapy through morbidity diagnosis. As mentioned above, conventional endoscopy and mucosal biopsy are not considered effective for EoEM diagnosis. Until now, surgical biopsy was required for the pathological diagnosis of the esophageal muscle layer, which is a highly invasive procedure, and has to be performed by a surgeon.

In 2010, peroral endoscopic myotomy (POEM) was developed, along with a POEM-enabled, less invasive, transoral approach for the esophageal muscle layer.^{10, 11} POEM was next developed into peroral esophageal muscle biopsy (POEM-b),¹² with the help of which EoEM can now be diagnosed (see Method sections for details). Eosinophil infiltration was

observed in both EoE and EoEM specimens, although the eosinophil-invaded location was different between these two diseases just as mucosal type and muscle layer type of EoGE.

While the exact pathological mechanism of EoE has yet to be elucidated, it is known that it is closely associated with Th2-mediated allergic reactions,¹³⁻¹⁵ and some of the cells and molecular players involved in the pathology have been identified. Mast cells were reported to be involved in EoE.¹⁶ Eotaxin-1 (CCL11) and eotaxin-3 (CCL26) are chemokines that are overexpressed under Th2-mediated allergic conditions. They strongly recruit Th2 allergy-related cells, such as eosinophils and mast cells, through the chemokine receptor CCR3, which is expressed on those cells.¹⁷⁻¹⁹ Eotaxin-1 has a critical role in regulating the circulating eosinophil levels in the healthy GI tract except the esophagus; whether it plays a role in pathological conditions is unknown. However, local production of eotaxin-3 by esophageal epithelia leads to eosinophil accumulation in EoE.²⁰ Although the eotaxin-CCR3 axis is considered a key pathway in the local Th2-reaction of EoE, knowledge on the immunopathology of EoE is limited, while the immunopathology of EoEM is completely unknown. Recent genome-wide association studies (GWAS), candidate gene, and mapping/sequencing studies²⁰⁻²⁴ have associated several genes, such as *CCL26* (encodes eotaxin-3), *DSG1* (encodes desmoglein-1), and *FLG* (encodes filaggrin), with EoE. Further, it is known that DSG1 and FLG are associated with the barrier function of the squamous

epithelium, and change in their expression levels could contribute to EoE pathogenesis. Therefore, the expression of the corresponding proteins should be investigated in EoE and EoEM to identify any potential differences. Consequently, in this study, we attempted to distinguish EoEM from EoE by immunohistochemical analysis.

PATIENTS AND METHODS

Patients

Three patients were diagnosed with EoEM in our case series July 2014 through January 2016. We previously reported the details of the clinical course of the first two cases.⁷ In the same period, three other patients were diagnosed with EoE, based on the recommendations by the American Gastroenterological Association.²⁵ The patient characteristics are shown in Table 1. Two full-layer, normal specimens from esophagectomies of two patients with esophageal squamous cell carcinoma (a 75-year-old man, and a 65-year-old man) were used as controls.

The present study was performed in accordance with the Declaration of Helsinki. The ethical committee approval of the hospital was obtained (No1917), and informed consent was obtained from all patients. This study was conducted as part of a clinical study registered with the University Hospital Medical Information Network (UMIN) (ID: UMIN 000018685).

POEM and POEM-b

Upon HRM, the three patients with EoEM were initially diagnosed as Jackhammer esophagus (two cases) and Nutcracker esophagus (one case), according to the Chicago classification criteria (Figure 1A).^{9, 26} In each case, six esophageal mucosal biopsies were performed to increase the detection rate of mucosal eosinophilia. However, there were no characteristic findings in the mucosal specimens that could lead to a specific diagnosis (Figure 1B; normal endoscopic finding in EoEM). The patients were first treated with calcium blockers and proton pump inhibitors; however, chest pain and dysphagia were not alleviated. Therefore, we performed POEM based on the technique described by Inoue et al.¹⁰ While incising the muscularis propria, full-thickness muscle layer biopsies (POEM-b) were performed using the ends of the biopsy forceps (Figure 1C). Several muscle layer samples from the medial to the distal esophagus were obtained in each case. The histopathology of the deep mucosa and submucosa can generally not be observed by conventional biopsy, including in our three EoEM cases; therefore, in the third EoEM case, a mucosal entry for POEM/POEM-b was created with a cap-fitted endoscopic mucosal resection (EMR) to examine the full-layer of the mucosa along with the submucosa (Figure 2A).²⁷ The mucosal entry was closed with hemostatic clips after POEM/POEM-b.

Eosinophil and Mast cell Assessment

Hematoxylin and eosin-stained slides for samples from all the patients were examined. The numbers of eosinophils in the mucosal and muscle layer specimens were counted per high-power field (HPF; 400× magnification); the maximum numbers were recorded. In the same way, the number of mast cells was counted by tryptase immunohistochemical staining. Formalin-fixed sections were deparaffinized with xylene and ethanol and incubated with anti-mast cell tryptase antibody (ab2378; Abcam, Tokyo, Japan) for 18 hours at 4°C. A biotin-conjugated Rabbit anti-mouse IgG polyclonal (1/1000) was used as the secondary antibody. The sections were observed with the help of a digital microscope camera (DP73, Olympus, Tokyo, Japan).

Immunofluorescence Analysis

Th2 pathway-related molecular expression was examined by immunofluorescent staining. We examined the expression of eotaxin-1, eotaxin-3, and CCR3 with goat polyclonal anti-eotaxin-1 (AF-320-NA; R&D, Minneapolis, USA), goat polyclonal anti-eotaxin-3 (500-P156G; Peprotech, Rocky Hill, USA), and rabbit monoclonal anti-CCR3 (ab32512; Abcam). Moreover, mouse monoclonal anti-desmoglein-1 (ab12077; Abcam), and

mouse monoclonal anti-filaggrin (ab17808; Abcam) antibodies were used for staining the esophageal epithelium to assess the expression levels of desmoglein-1 and filaggrin in EoE and EoEM and to identify potential differences. Changes in gene expression levels of these two molecules have been previously reported for EoE.^{21, 24} Sections were stained with the secondary antibodies Alexa Fluor 555-labeled donkey anti-goat IgG, Alexa Fluor 488-labeled chicken anti-rabbit IgG, and Alexa Fluor 594-labeled goat anti-mouse IgG and counterstained with 4',6-diamidino-2-phenylindole (DAPI; mounting medium with DAPI; Vector Laboratories Inc., Burlingame, CA, USA). The sections were observed by immunofluorescent microscopy (BZ-9000; KEYENCE, Osaka, Japan).

Expression of each molecule (other than desmoglein-1 and filaggrin) was observed using 400× magnification, and only that together with nuclear staining was considered positive. The esophageal epithelium was graded on a four-point scale as follows: -, never observed; ±, faint staining; +, patchy or focal distribution of positive cells (less than 50 % positivity in the esophageal epithelium); #, dense distribution of positive cells (more than 50 % to full-layer positivity). Similarly, the esophageal muscle layers were graded as: -, never observed; ±, faint staining; +, patchy or focal distribution of positive cells (less than 50 % positivity/HPF); and #, dense distribution of positive cells (more than 50 % positivity/HPF). For the +/# category, the positive cell types were described. The expression of desmoglein-

1 and filaggrin was graded as normal (same as control, entire epithelial cell membrane is stained), or showing mild (50–75 % of the membrane was stained), moderate (25–50 % of the membrane was stained), and severe (0–25 % of the membrane was stained, totally disrupted membrane staining) disruption.

The histopathological assessment was performed by two doctors (a physician and a pathologist) together who were blind to the clinical data.

RESULTS

Mucosal and muscle-layer specimens of the three EoEM patients were obtained. Mucosal biopsy specimens of the three EoE patients were also collected. These examinations were compared to two full-layer, non-carcinoma esophageal slices extracted from two esophageal squamous cell carcinoma patients as controls. (Table 2)

Histopathology of EoEM and EoE

For the three EoEM patients, muscle-layer biopsy by POEM-b revealed eosinophilic infiltration in the perivascular and perimysial areas in the esophageal muscle layer (Figure 2B). At 400× magnification, we detected invasion by a maximum of 30, 36, and 73 (number/HPF) eosinophils in each case (Figure 2C). Such severe eosinophil infiltration was

observed in 6/6, 6/6, and 4/5 muscle biopsy specimens in the three cases, respectively. Therefore, the eosinophil detection rate of POEM-b was calculated as 94.1 % (16/17). In contrast, no eosinophil infiltration was observed in the esophageal mucosa and submucosa of the EoEM samples. Only intraepithelial capillary extension and cytoplasmic vacuolation were observed in the basal layer of the epithelia in those samples (Figure 2A). Severe mast cell invasion was also only observed in the esophageal muscle layer (a maximum of 12, 9, and 5/HPF, respectively) (Figure 2C, insert), not in the epithelium (1, 0, and 1/HPF, respectively).

The histopathology of the esophageal epithelium in the EoE samples was remarkably different from that in the EoEM samples. A number of eosinophils (a maximum of 50, 44, 40/HPF, respectively) were observed in the epithelium with dilated intercellular spaces, basal-zone hyperplasia, and papillary elongation (Figure 3C). Mastocytosis was observed in the esophageal epithelium (a maximum of 15, 14, and 4/HPF, respectively) of EoE (Figure 3C, insert).

Immunofluorescence Analysis of EoEM Samples

In the esophageal muscle layer of EoEM, the numbers of CCR3-positive infiltrating cells increased in the perivascular and perimysial area where eosinophil infiltration was observed (CCR3 Grading; +/+/+ [Figure 2D]). Eotaxin-1 and Eotaxin-3 were positive for

smooth muscle cells and vascular endothelial cells in the muscle layer (Eotaxin-1 Grading; #/#/# [Figure 2D], Eotaxin-3 Grading; #/#/#, respectively).

On the other hand, in the esophageal mucosa of EoEM (all cases, six conventional biopsies; third case, additional full mucosa and submucosa samples obtained by EMR), CCR3 -positive cells were never observed (CCR3 Grading; -/-/-). Eotaxin-1- and -3-positive cells were focally distributed to the peripapillary and basal layer of the epithelium (Eotaxin-1 Grading; +/+/, Eotaxin-3 Grading; +/+/, respectively). In the mucosal and submucosal sample of the third case, eotaxin-1 and eotaxin-3 were also positive in the vasculature and muscularis mucosae. In the two control slides, CCR3-, eotaxin-1-, and eotaxin-3 -positive cells were never seen both in the mucosal and muscle layer.

The expression of desmoglein-1 in each of the patients was similar to that in the control (Grading; all normal) (Figure 4).

Immunofluorescence Analysis of EoE Samples

In the esophageal epithelium of EoE, eotaxin-3 was clearly overexpressed in the epithelial cells throughout the epithelial layer (Grading; #/#/#), while CCR3 was overexpressed in the infiltrating cells (Grading; +/+/) (Figure 3D). Moreover, the number of eotaxin-3-positive cells was significantly higher than that of eotaxin-1-positive cells

(Grading; $\pm/\pm/\pm$) in EoE. Desmoglein-1 in all the three patients was severely downregulated in the epithelial layer (Grading; all severe) (Figure 4).

Although we attempted immunofluorescence analysis of filaggrin, we were unable to detect its expression in our case series. This indicates that the *FLG* gene for filaggrin is not expressed in the esophageal squamous epithelium, and that changes in the expression level of this gene are not directly involved in esophageal barrier dysfunction in EoE and other EoGIDs.²⁸ Additionally, the use of filaggrin seems unlikely to be useful given that the esophagus is at best partially keratinized.

DISCUSSION

Here, we investigated the immunopathology of EoEM in order to differentiate it from EoE.

EoE has been reported as a Th2-mediated allergy disease of the esophageal epithelium that is associated with antigen.¹⁵ In our EoE case series, the expression of eotaxin-3 was increased in the esophageal epithelium, while that of the epithelial barrier molecule desmoglein-1 was downregulated. Therefore, our results corroborate those reported by previous studies. In our study, the expression of eotaxin-3 in the esophageal epithelium was also significantly higher than that of eotaxin-1. This chemokine milieu attracts infiltrating

cells, such as eosinophils and mast cells, through their chemokine receptor CCR3. These results suggest the following mechanism for EoE development: epithelial barrier dysfunction, as indicated by reduced desmoglein-1 levels, facilitates allergen entry into the esophageal epithelium; this leads to a local Th2 reaction. Increased eotaxin-3 levels recruit eosinophils and mast cells through CCR3.²⁹

The esophageal epithelium of EoEM expressed high levels of desmoglein-1, which indicates intact cell-cell junctions—different from what we observed in EoE. Eotaxin-3, which is significantly overexpressed in the epithelium of EoE, was expressed only in the esophageal peripapillary in the epithelium of EoEM. Eotaxin-1 had similar distribution as Eotaxin-3 in the EoEM epithelium. This characteristic eotaxin-3 and eotaxin-1 distribution may indicate intraepithelial capillary extension in histopathology. CCR3 was “never observed” in the epithelium, which indicates that the pathology of EoEM does not involve Th2-mediated allergic reactions in the esophageal epithelia unlike EoE. Eotaxin expression on smooth muscle cells and vascular endothelial cells was previously reported.³⁰⁻³² In our case series, we also observed eotaxin-1 and -3 expression on the smooth muscle and vascular endothelial cells, and the role of eotaxin-1 appears to be superior to that of eotaxin-3, different from that in the esophageal epithelium in EoE. Therefore, we hypothesize that EoEM is a new type of EoGID, with a mechanism different from that of EoE. The

pathologically high eotaxin-1 expression in EoEM might indicate that EoEM has a systemic pathogenesis because eotaxin-1 has a critical role in regulating the circulating eosinophil levels in the healthy GI tract except the esophagus, and is not an esophageal localized disease like EoE, which has an eotaxin-3-dominant pathology. Additionally, mast cells infiltrating the esophageal smooth muscle and their degranulation could potentially increase smooth muscle contraction.³³

One important question is: how can EoEM be differentiated from EoE and from esophageal motility disorders in the clinic? Our case series study indicates that patients with EoEM show strong contraction of the esophageal body upon HRM, probably because inflammatory responses by eosinophils in the proper muscle layer activate smooth muscle contraction.³⁴⁻³⁶ In contrast, patients with EoE had failed peristalsis on HRM in our case series. Esophageal motility dysfunction in EoE is probably due to mucosal chronic fibrotic change. HRM is a useful tool to raise a first suspicion of EoEM. In addition, high peripheral IgE levels indicate immunological sensitization for an allergen and are elevated in EoGIDs. However, they are considered not to be mediated by IgE.^{36, 37} Thus, increased IgE levels may be used as a further indicator of EoEM in esophageal symptomatic patients. In the three EoEM cases in this study, severe eosinophil infiltration was seen in a focal distribution, and was only found in the perimysial and perivascular area and was not diffusely distributed on

the proper muscle layer. Since this uneven distribution could lead to a false negative diagnosis, several biopsies should be performed for an accurate diagnosis. Here, almost all muscle specimens in our series showed severe eosinophil infiltration (eosinophil detection rate of POEM-b: 94.1%) which indicates POEM-b is a feasible way to investigate the eosinophil invasion in the muscle layer of EoEM.

It is important to note that this study also has some limitations. For example, we considered few cases of EoGIDs, and used only limited antibody types in our immunofluorescence protocol. Future research should be directed at the triggers for EoEM other than epithelial barrier dysfunction.

In conclusion, eotaxin-1 and eotaxin-3 expression on the smooth muscle and blood vessels play a role in the pathogenesis of EoEM. EoEM has some unique immunological features that distinguish it from EoE.

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Table 1. Characteristics of patients enrolled in this study.

Case No.	1	2	3	4	5	6	7	8
Diagnosis	Eosinophilic esophageal myositis			Eosinophilic esophagitis			Control (esophageal cancer)	
Age	43	31	74	54	31	54	75	65
M/F	M	M	M	M	M	F	M	M
Allergy history	no	no	no	no	House dust	Milk		
Eos (/μL) [†]	388.1	80.6	253.7	109.8	499.5	212.7		
(%)	(6.6)	(2.1)	(2.8)	(2.2)	(7.5)	(3.1)		
IgE IU/mL [‡]	194.0	368.0	192.0	33.3	314.0	33.5		
Motility diagnosis [§]	Jackhammer esophagus	Nutcracker esophagus	Jackhammer esophagus	Failed peristalsis	Failed peristalsis	Failed peristalsis		

[†]Eos: peripheral blood eosinophils (normal range 0.2%-8.4 %), [‡]IgE: peripheral IgE level (normal range ≤ 173 μL)

[§]According to the Chicago classification criteria (before October 2014: earlier (2011) version⁹, and after: v3.0²⁶).

Table 2: Summary of histopathological and immunopathological findings for eosinophilic gastrointestinal diseases

			Number of Eosinophil	Number of Mast cell	Grading ^s			
					CCR3	Eotaxin-3	Eotaxin-1	Desmoglein-1
EoEM [†] (n=3)	esophagus	Epithelium	0, 0, 0	1, 0, 1	-/-/-	+ / + / + (peripapillary and basal layer of the epithelium)	+ / + / + (peripapillary and basal layer of the epithelium)	normal/normal/normal
		Muscle layer	30, 36, 73	12, 9, 5	+ / + / + (infiltrating cells)	# / # / # (vessels, smooth muscle)	# / # / # (vessels, smooth muscle)	not analyzed
EoE [‡] (n=3)	esophagus	Epithelium	50, 44, 40	15, 14, 4	+ / + / + (infiltrating cells)	# / # / # (epithelium)	± / ± / ±	severe/severe /severe
Control (n=2)	esophagus	Epithelium	0, 0	1, 0	-/-	-/-	-/-	normal/normal
		Muscle layer	0, 0	2, 2	-/-	-/-	-/-	not analyzed

[†]Eosinophilic esophageal myositis. [‡]Eosinophilic esophagitis.

Distribution of positive cells is indicated in parenthesis

Grading^s (CCR3, Eotaxin-3, Eotaxin-1)

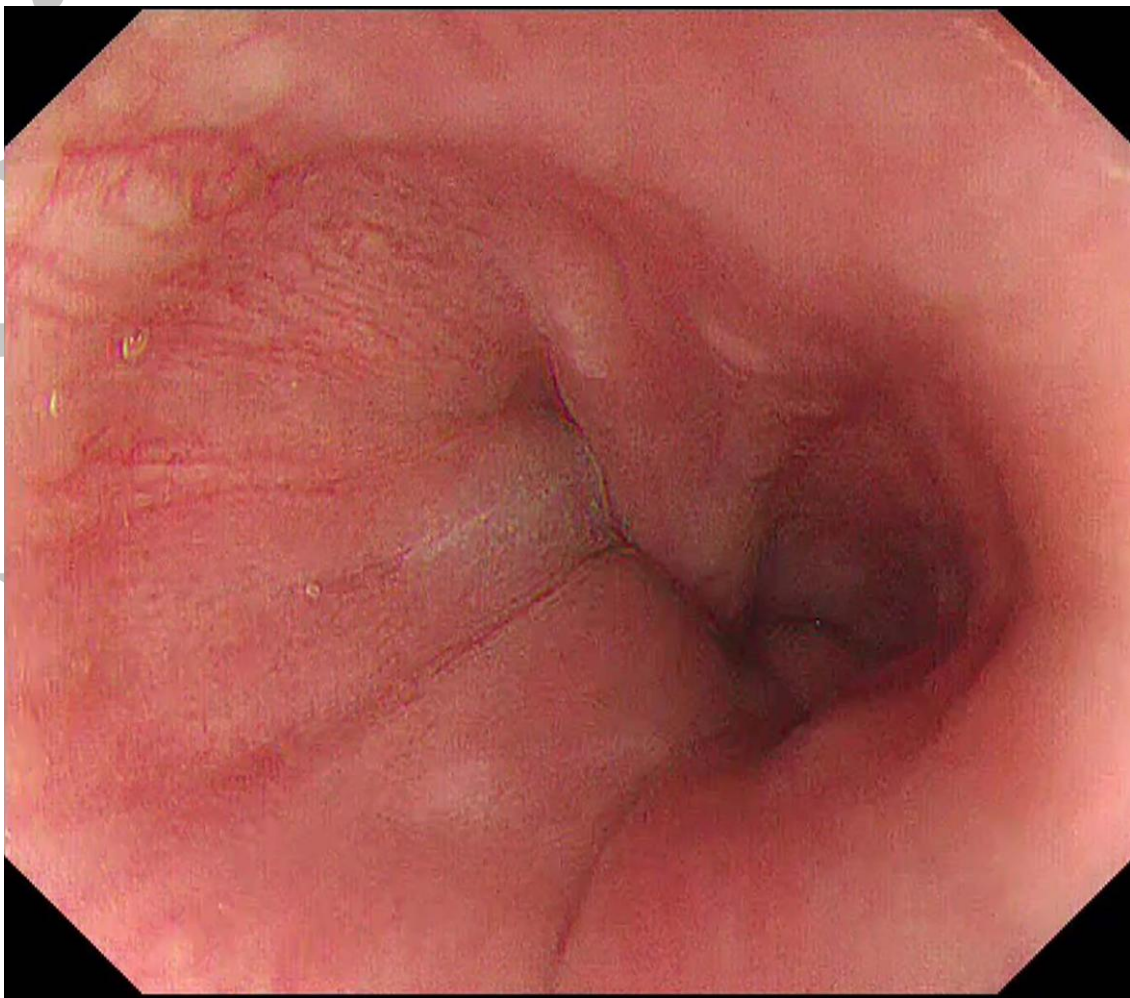
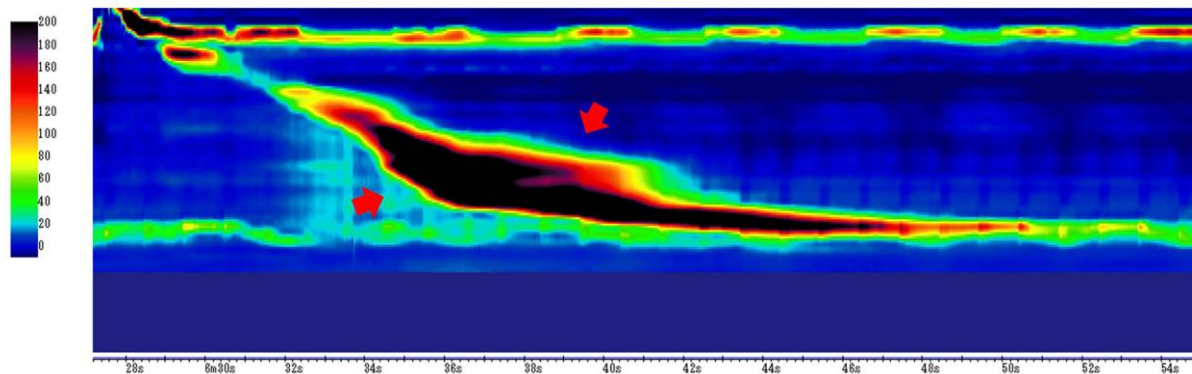
Esophageal epithelium: -: never observed, ±: faint staining, +: patchy or focal distribution of positive cells, #: dense distribution of positive cells (more than 50 % to full layer positivity)

Esophageal muscle layer, ±: faint staining, +: patchy or focal distribution of positive cells, #: dense distribution of positive cells (more than 50% positivity/HPF)

Grading^s (Desmoglein-1)

normal (same as control/ entire epithelial cell membrane is positive), mild (50-75 % of the membrane was stained), moderate (25-50 % of the membrane was stained), and severe (0–25 % of the membrane was stained/totally disrupted membrane staining)

FIGURE LEGENDS



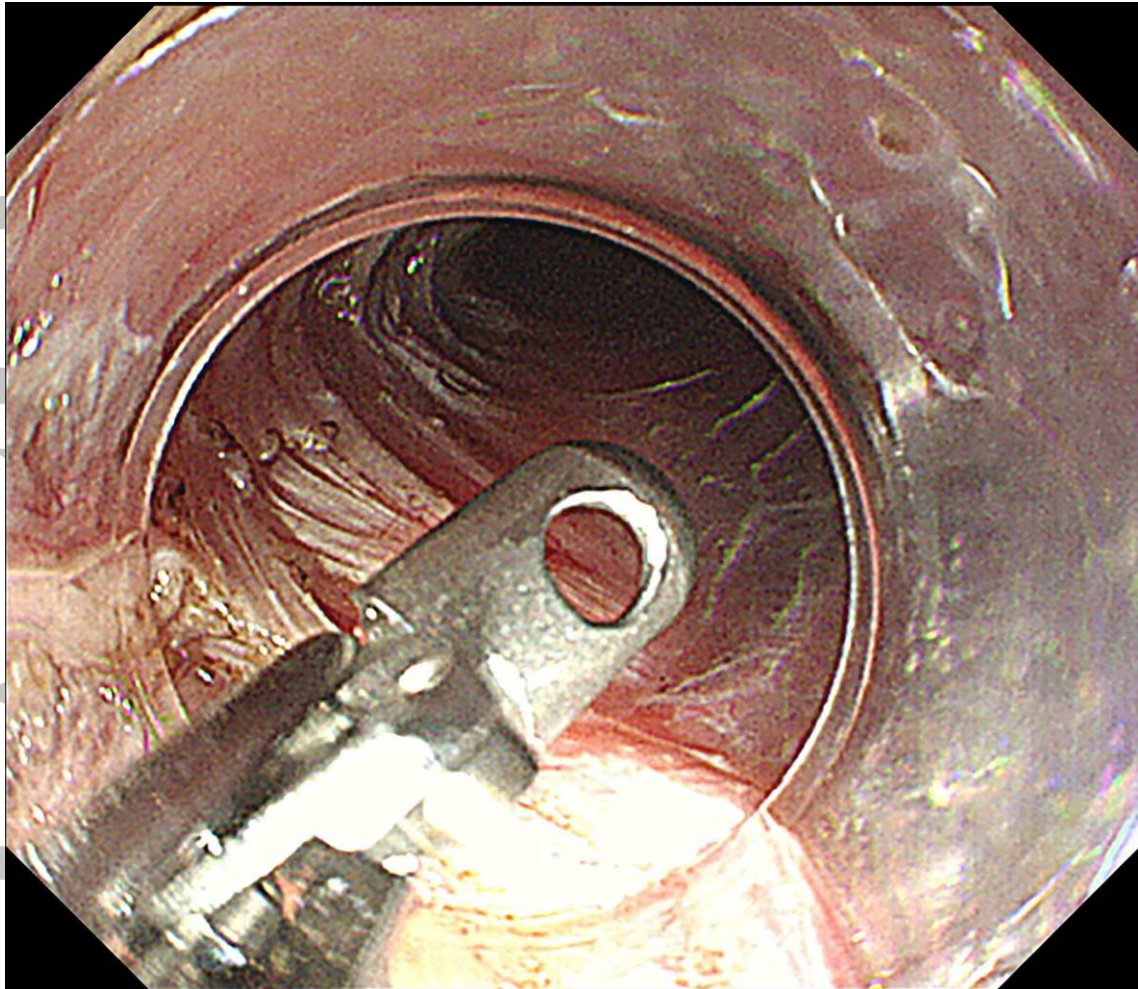
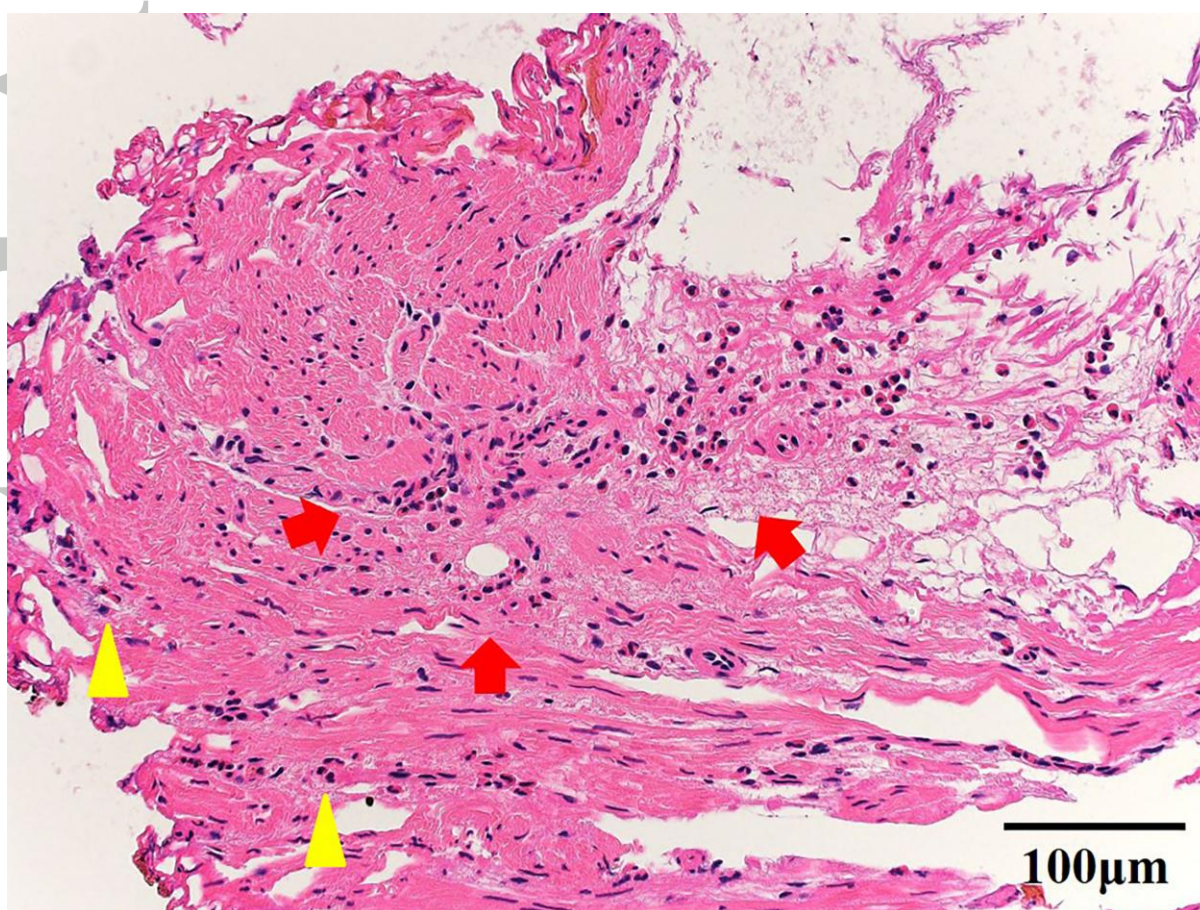
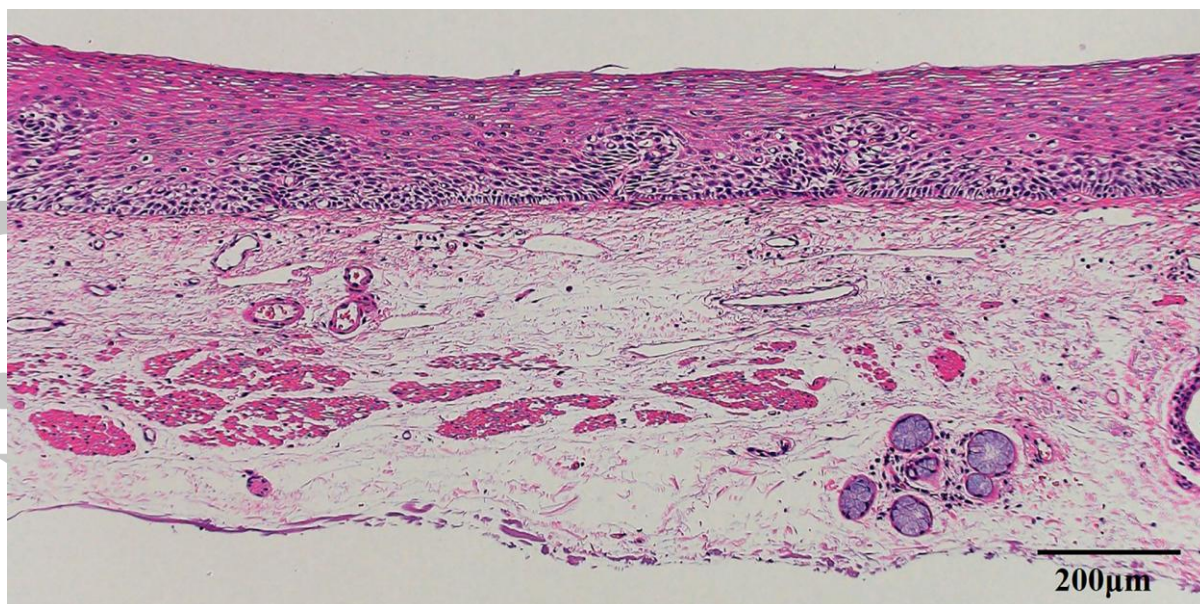
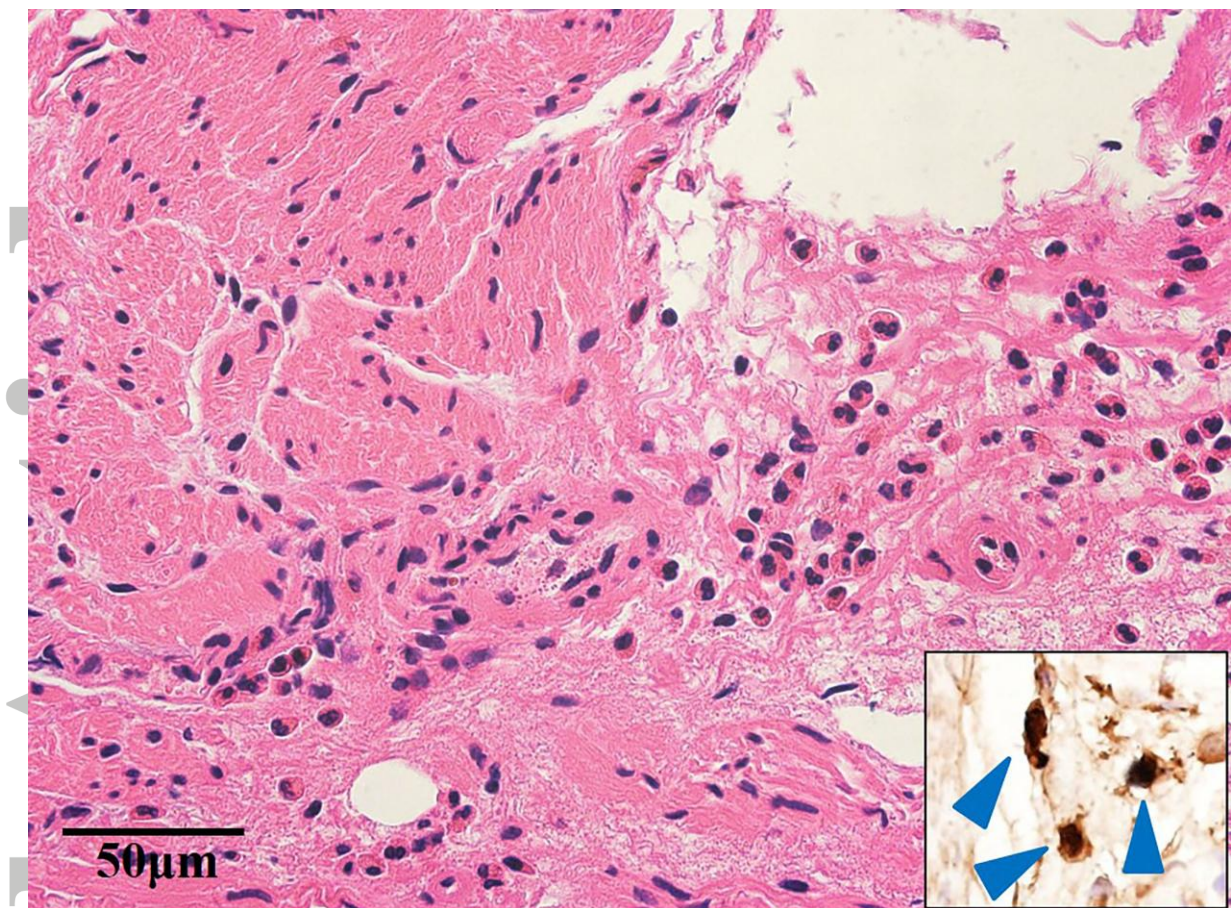


Figure 1. (A) High-resolution manometry (HRM) results of the third case of eosinophilic esophageal myositis (EoEM) in this study (the first two have been reported previously). The results show extremely high maximum distal contractile integral (DCI) (19814.9 mmHg/[s·cm], red arrows). The patient was diagnosed with jackhammer esophagus, based on Chicago classification criteria.

(B) Symptomatic patients diagnosed with Jackhammer and Nutcracker esophagus upon HRM showed normal mucosa on conventional endoscopy. (C) Peroral esophageal muscle biopsy (POEM-b) was performed, and EoEM was diagnosed based on eosinophil infiltration localized in the muscle layer of the esophagus.





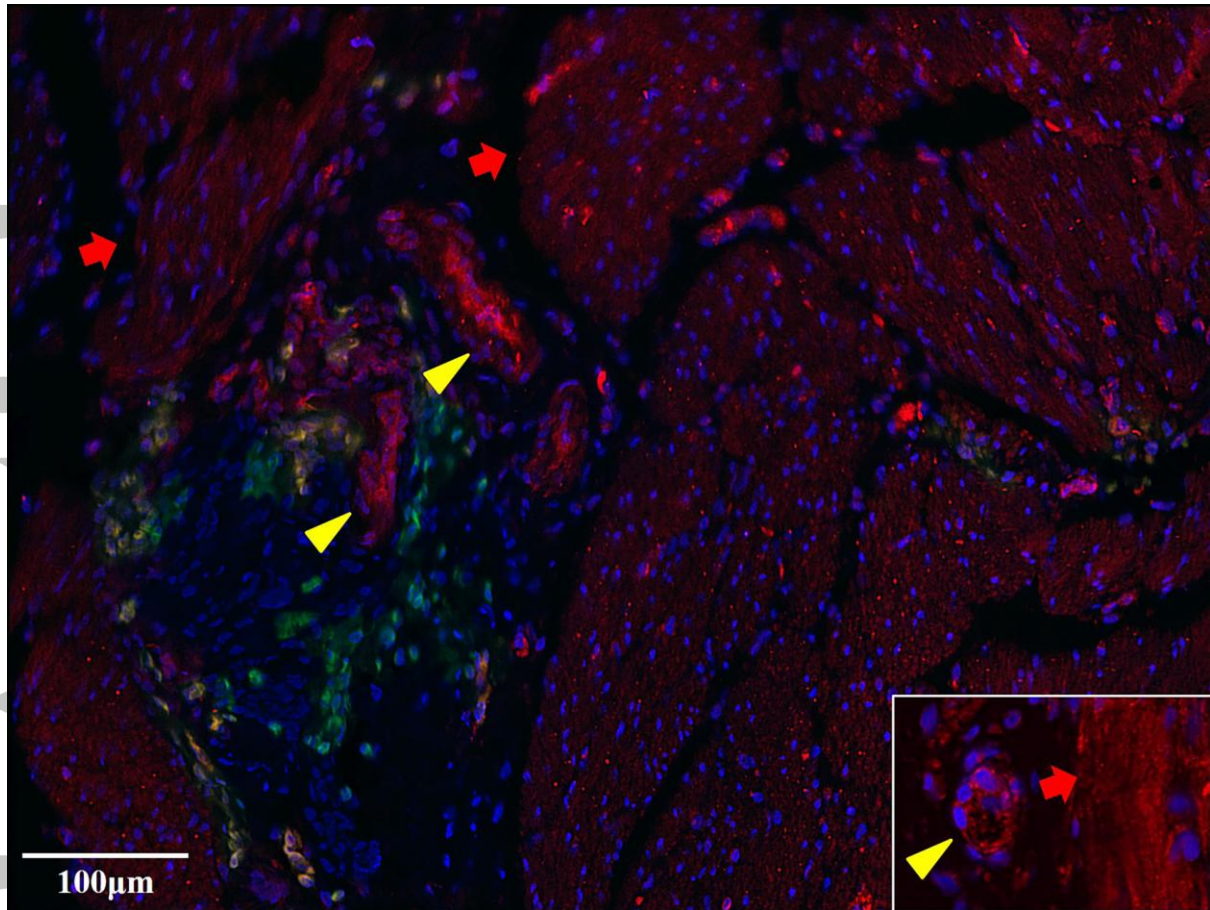
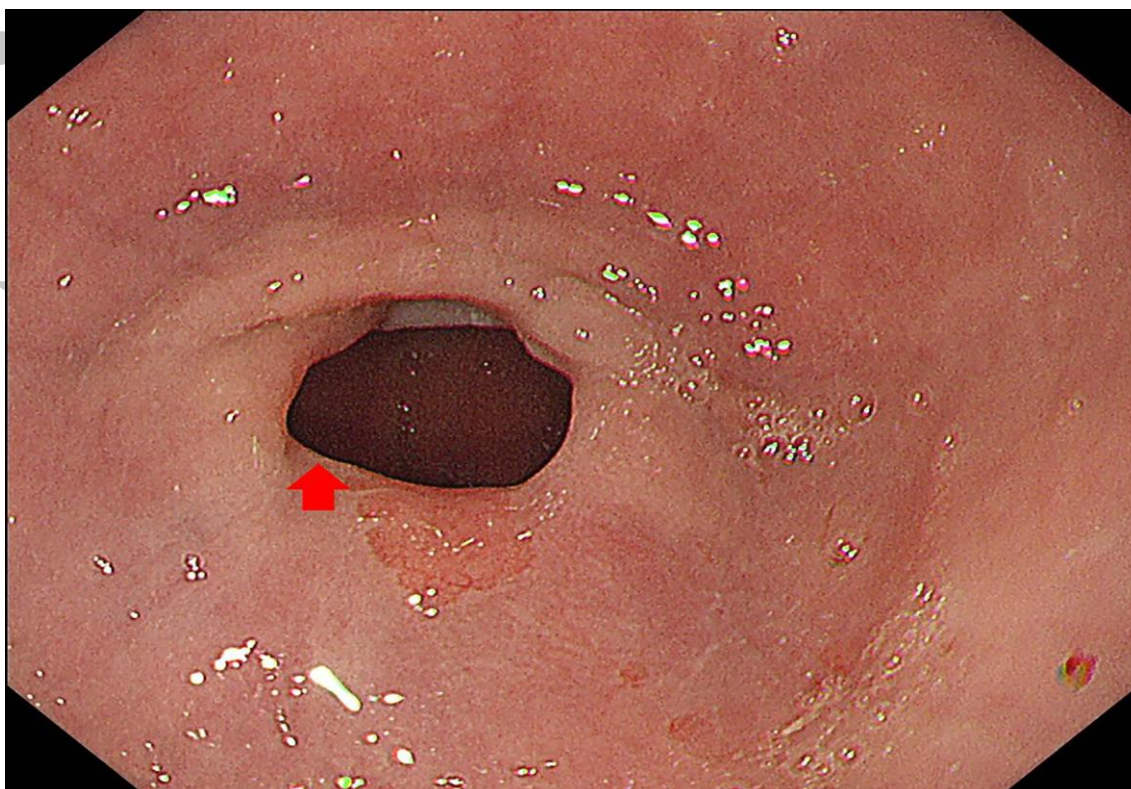
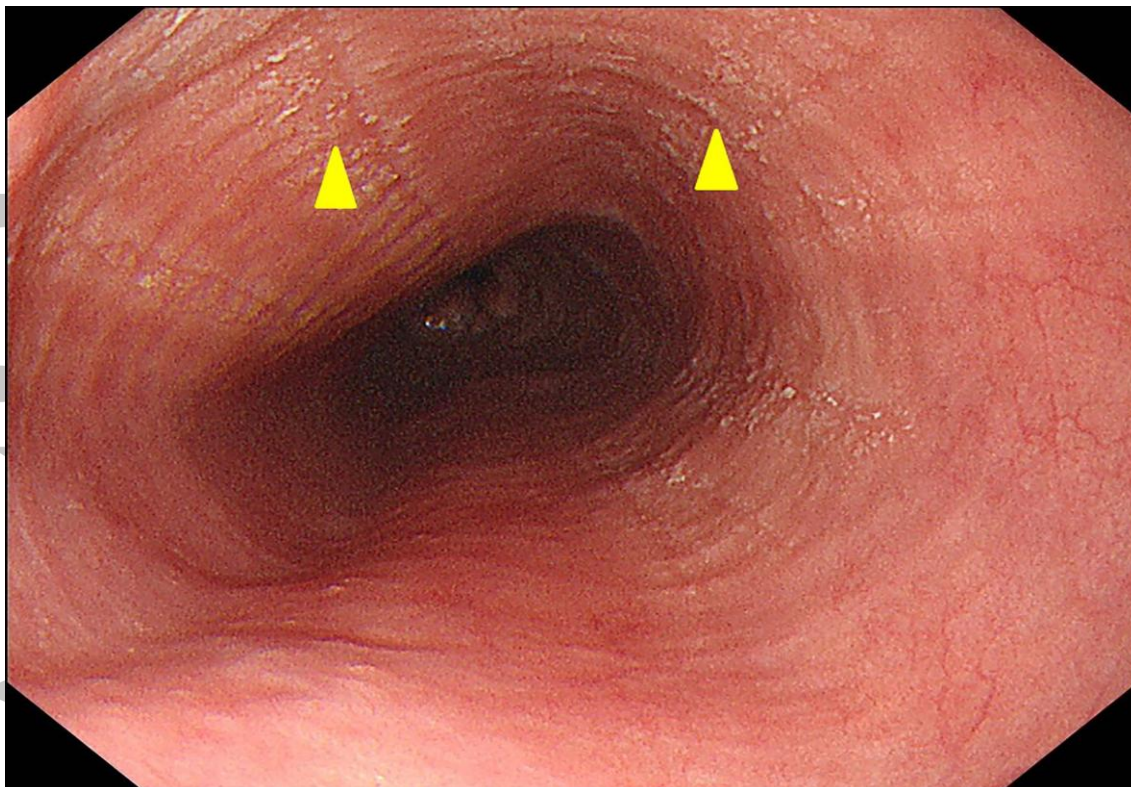
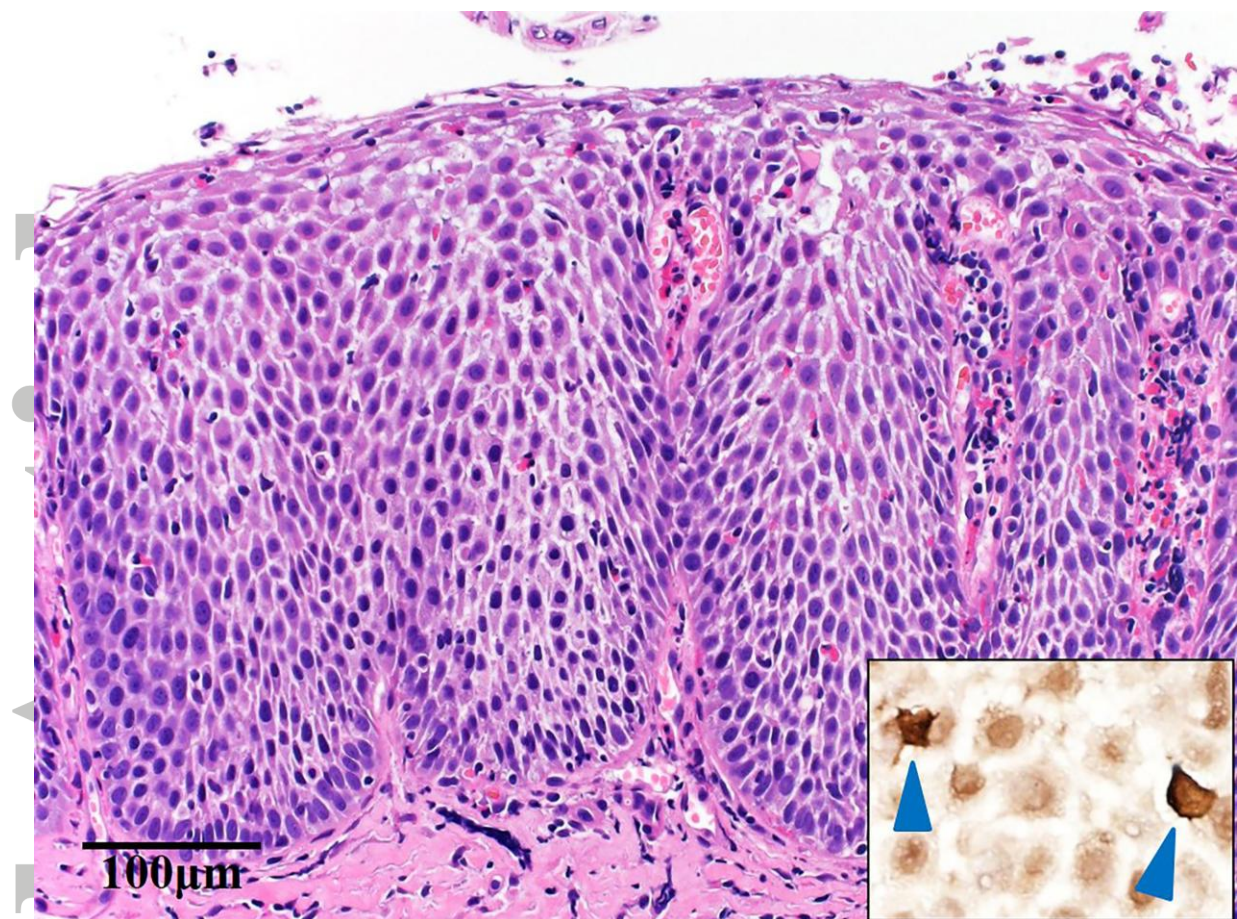


Figure 2. Histopathology and immunofluorescent analysis of EoEM.

In addition to six mucosal biopsies for the third case of EoEM, entry for POEM-b was created by cap-endoscopic mucosal resection method in order to obtain mucosal and submucosal specimens. (A) No characteristic findings were noted in the mucosa and submucosa, except for slight hyperplasia of the epithelium. (B) Eosinophilic infiltration was seen in the perivascular and perimysial area of the muscle specimens at 200× magnification. Yellow triangles and red arrows show the location of eosinophilic infiltration. (C) Magnified (400×) image for the area indicated by red arrows in (B). (C, insert) Severe mast cell invasion (blue triangles) was also observed in the perimysial areas of esophageal muscle layer.

(D) Immunofluorescent double staining for CCR3 (green) and eotaxin-1 (red) in EoEM, at 200× magnification. Inset shows the image at 400× magnification. The infiltrating cells were positive for CCR3. Myocytes (red arrows) and vascular endothelial cells (yellow triangles) were positive for eotaxin-1.





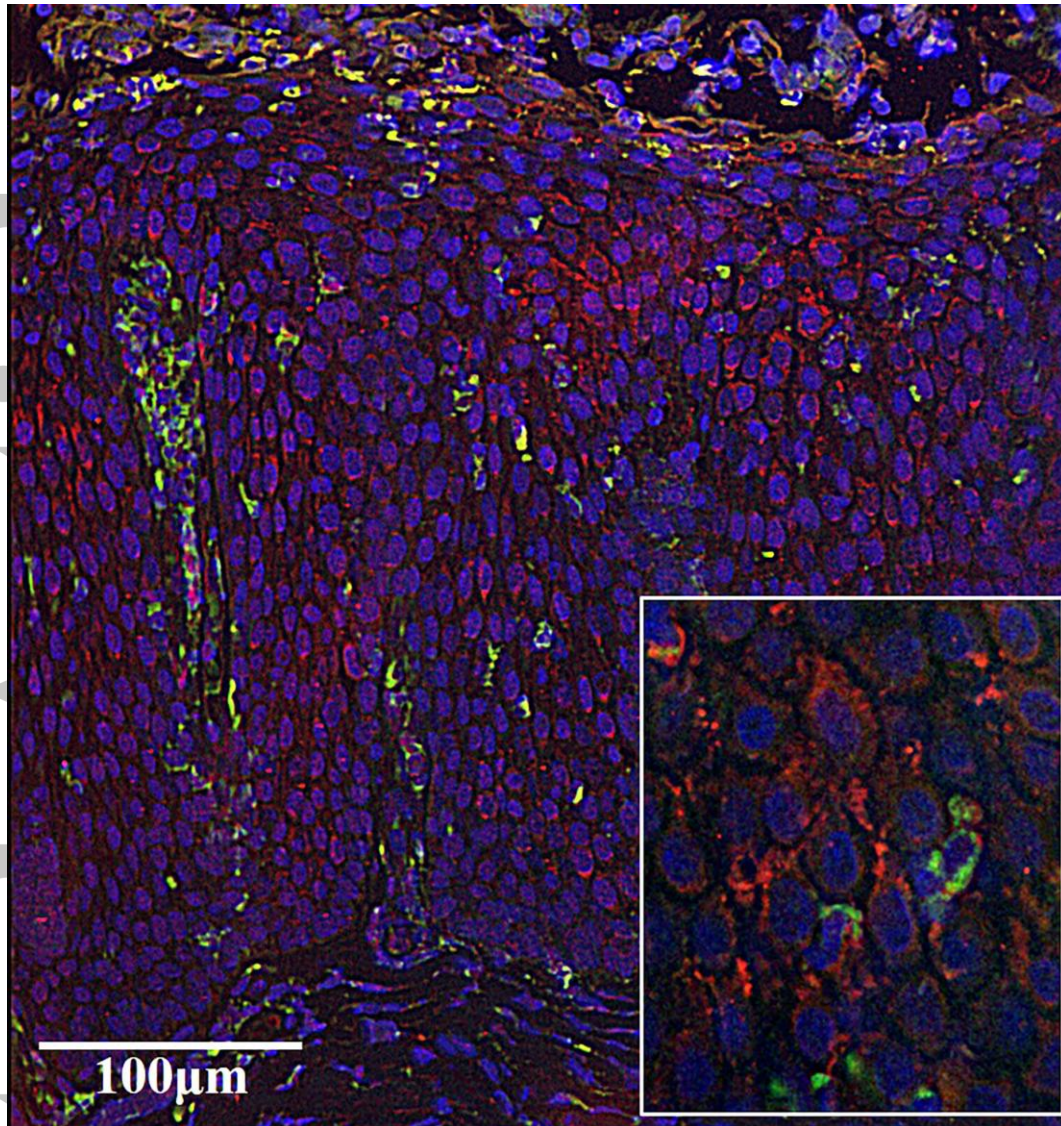


Figure 3. In eosinophilic esophagitis (EoE), endoscopy shows loss of vascularity, white plaques, and longitudinal furrows (yellow triangles) in the medial esophagus (A) and presence of fixed rings in the lower esophagus (B; red arrow). (C) Significant eosinophil inflammation (50 eosinophils per high-power field) was seen in esophageal epithelium of EoE, at 200× magnification. (C, insert) Mastocytosis (blue arrows) was observed in the esophageal epithelium of EoE.

(D) Immunofluorescent double staining for CCR3 (green) and eotaxin-3 (red) in EoE, at 200× magnification. Inset shows the image at 400× magnification. CCR3-positive cells infiltrated the eotaxin-3-positive epithelial cells.

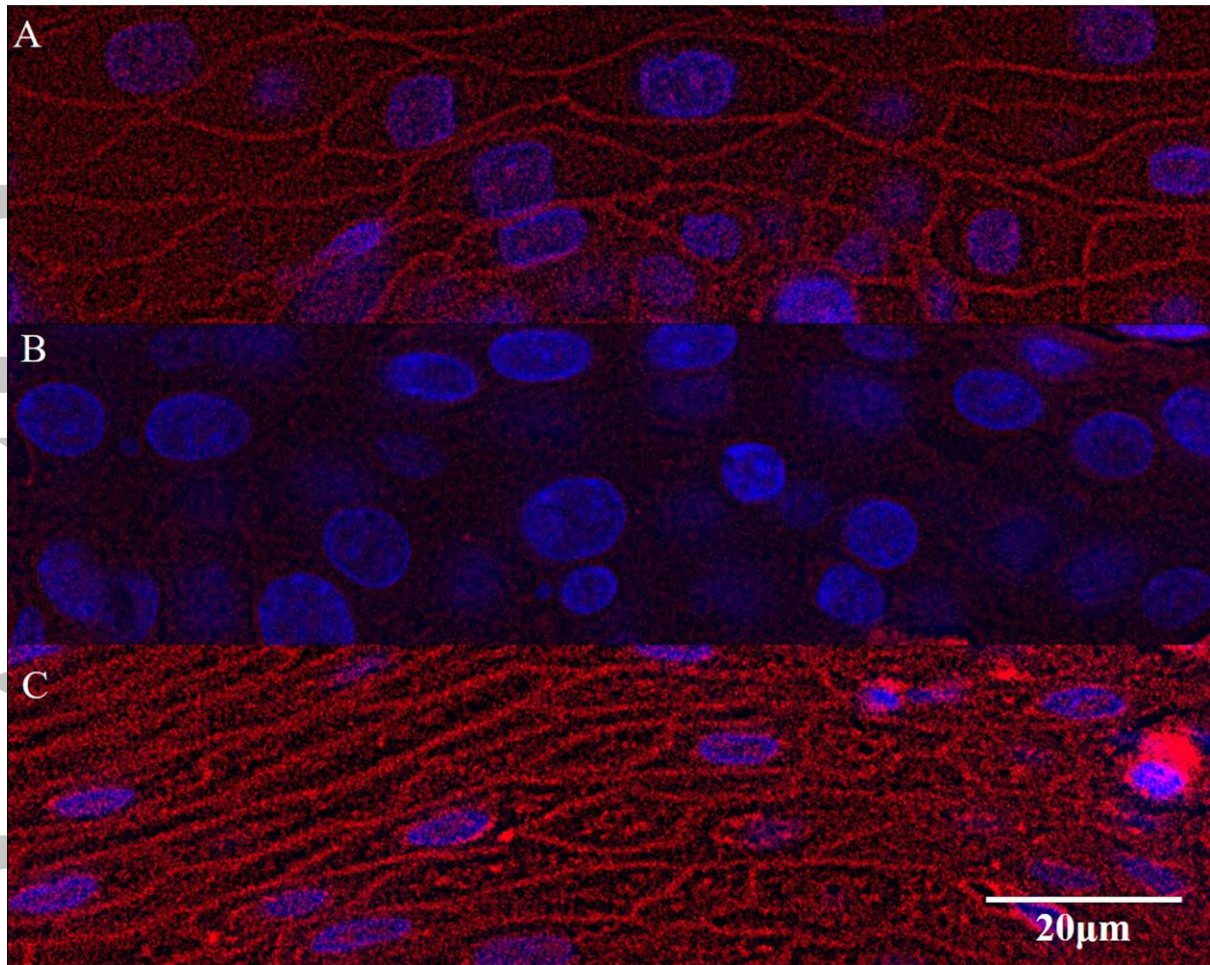


Figure 4. Immunofluorescence for desmoglein-1 (red) in esophageal biopsy samples of patients with EoEM (A), EoE (B), and control (C), at 1000× magnification. Desmoglein-1 expression was clearly maintained on the esophageal epithelial cell membrane in control (C) as well as in EoEM (A). In contrast, in EoE, expression of desmoglein-1 was significantly lost in the epithelial layer of the esophagus (B).