gastrointestinal disorders

MDPI

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The Predictive Factors of Responsiveness to Proton Pump Inhibitor Therapy for Eosinophilic Esophagitis

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Received: 10 January 2019; Accepted: 21 February 2019; Published: 25 February 2019

Abstract: Approximately half of patients with eosinophilic esophagitis (EoE) respond clinically and histologically to proton pump inhibitor (PPI) therapy. Although recent guidelines suggest that PPI-responders and non-responders were included in EoE, it is important to investigate the predictive factors of PPI- responsiveness. This study aimed to determine the rate of PPI- responders and compare the characteristics of PPI-responders and non-responders. Fifty-nine patients with esophageal eosinophilia received PPI therapy for eight weeks, and its efficacy was assessed. PPI- responsiveness was diagnosed based on the relief in symptoms and reduction of intraepithelial eosinophilic infiltration to <15 per high-power field (hpf) after PPI therapy. Multivariate analysis was performed to identify factors associated with PPI-responders. Of the 59 patients, 41 (69.5%) were diagnosed with PPI-responders. The rate of gastrointestinal (GI) screening in the indications for endoscopy was significantly higher in patients with PPI- responders than in those with non-responders. On multivariate analysis, GI screening and presence of reflux esophagitis was associated with an increased odds ratio (OR) of PPI-responders, but presence of rings with a decreased OR of PPI-responders. Presence of reflux esophagitis and absence of rings on endoscopy especially during GI screening might be significant predictive factors for PPI response in patients with EoE.

Keywords: esophagus; eosinophilic esophagitis; proton pump inhibitor

1. Introduction

Eosinophilic esophagitis (EoE) is a chronic, immune-mediated disease that might occur due to exposure to food and/or aeroallergens [1,2]. EoE is defined as presence of esophageal symptoms such as dysphagia and food impaction, as well as intraepithelial eosinophil infiltration of ≥15 per high power field (hpf) on esophageal mucosal biopsies, and is usually diagnosed based on the typical endoscopic findings [3]. Although EoE is uncommon in Asia compared to Western countries, the number of cases has been increasing in Japan [4–7].

Proton pump inhibitor-responsive esophageal eosinophilia (PPI-REE) is a defined entity that has clinical symptoms and histological findings similar to those of EoE but responds positively to PPI therapy in terms of clinical and histological outcomes [8]. The American College of Gastroenterology (ACG) guideline proposed that PPI-REE should be distinguished from EoE [9], whereas a recent

guideline and consensus suggested that PPI-REE be included in EoE [10,11]. Several studies analyzed differences between PPI-responders (PPI-REE) and non-responders, but most failed to find any significant differences [12–15].

However, the identification of factors associated with a response to PPI in patients with symptomatic esophageal eosinophilia could help in providing optimal treatment and contribute to the understanding of the pathological difference between PPI-responders and non-responders. This observational study aimed to determine the rate of PPI-responders and compare the clinical characteristics and histological and endoscopic findings between PPI-responders and non-responders in Japanese patients with symptomatic esophageal eosinophilia.

2. Results

2.1. Study Subjects

Of the 78 patients with esophageal eosinophilia, 16 were excluded because of lack of symptoms (six cases); other causes (six cases), including pemphigus (one case), autoimmune esophagitis (one case), eosinophilic gastroenteritis (four cases); and steroid use (five cases: three with steroid inhalation and two with systemic steroid therapy). One case was duplicated (other causes and steroid use). Among the 62 patients who received the PPI therapy, three were lost to follow-up and 59 completed the treatment and were analyzed (Figure 1). There were 39 men and 20 women with a median age of 46 years. The most frequent symptom was dysphagia (45 cases, 76.3%). No cases with food impaction were observed. An elevated eosinophil count was seen on blood investigations in 19 cases (34.5%), and the median eosinophil percentage was 5.3%. Forty-two (71.2%) patients had allergic diseases, including 11 cases with asthma, 27 with rhinitis, seven with dermatitis, and 13 with food allergy. The types of food allergies were as follows: egg, seafoods, fruits, cucumber, soba, and almond. The most frequent endoscopic finding was linear furrows (54 cases, 91.5%), and there were no cases with strictures. Examination of the biopsy specimens revealed that the median maximum intraepithelial eosinophil count was 40 per hpf.

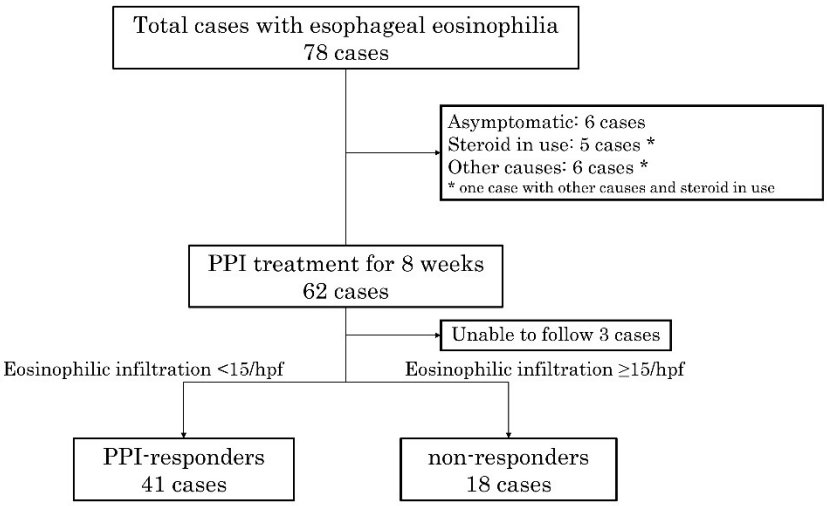

Figure 1. Patient flow. Definition of PPI-responsiveness.

2.2. Endoscopic Agreement

The kappa coefficient was 0.79 for linear furrows, 0.73 for rings, 0.66 for white plaques, 0.83 for reflux esophagitis, 0.43 for hiatus hernia, and 0.72 for atrophic gastritis.

2.3. Rate of PPI-Responders and Non-Responders and Their Characteristics

Of the 59 patients, 41 (69.5%) were diagnosed with PPI-responders and 18 (30.5%) with non-responders (Figure 2). The clinical characteristics of the patients diagnosed with PPI-responders and non-responders are shown in Table 1. Regarding the indications for endoscopy, the rate of GI screening in PPI-responders was significantly higher than in non-responders (68.3% in PPI-responders versus 33.3% in non-responders, $p = 0.021$). Dysphagia and food allergy in non-responders were higher than that in PPI-responders (70.7% in PPI-responders versus 88.9% in non-responders, $p = 0.189$; 17.1% in PPI-responders versus 33.3% in non-responders, $p = 0.187$). Improvement of symptoms was seen in 4 non-responders after PPI therapy, but none had complete resolution, and intraepithelial eosinophilic infiltration remained. However, there were no significant differences in these two factors, as well as in age, sex, BMI, smoking and drinking habits, duration of illness, and eosinophil counts, between PPI-responders and non-responders. The endoscopic findings are summarized in Table 2. The prevalence of reflux esophagitis was significantly higher in PPI-responders than in non-responders (41.5% in PPI-responders versus 5.6% in non-responders, $p = 0.006$). Of the 18 cases with reflux esophagitis, 17 (94.4%) cases were classified as grade A or B. Furthermore, the prevalence of rings was higher in non-responders than in PPI-responders (29.3% in PPI-responders versus 61.1% in non-responders, $p = 0.041$). There were no differences in other endoscopic findings, such as linear furrows, white plaques, edema, hiatus hernia, and atrophic gastritis, between PPI-responders and non-responders. The PPI therapy relieved reflux esophagitis in all cases. Although it is not shown in table, 11 cases are "ring" negative and "reflux esophagitis" positive, 6 cases are "ring" positive and "reflux esophagitis" negative, 6 cases are "ring" positive and "reflux esophagitis" positive, and 18 cases are "ring" negative and "reflux esophagitis" negative in PPI responders. No cases are "ring" negative and "reflux esophagitis" positive, 10 cases are "ring" positive and "reflux esophagitis" negative, 1 case is "ring" positive and "reflux esophagitis" positive, and 7 cases are "ring" negative and "reflux esophagitis" negative in non-responders. The rate of "ring" negative and "reflux esophagitis" positive in PPI-responders was significantly higher than in non-responders (26.8% in PPI-responders versus 0% in non-responders, $p = 0.013$), and the rate of "ring" positive and "reflux esophagitis" negative in PPI-responders was significantly lower than in non-responders (14.6% in PPI-responders versus 55.6% in non-responders, $p = 0.003$).

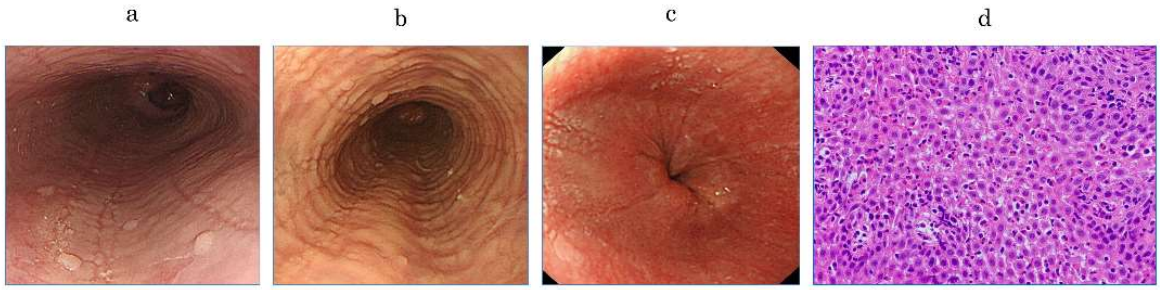

Figure 2. Endoscopic and histologic findings in patients with eosinophilic esophagitis (EoE). Typical endoscopic findings such as (**a**) linear furrows, (**b**) rings, and (**c**) white plaques in PPI-responders and non-responders. Eosinophil infiltration is observed in the esophageal epithelium (**d**).

2.4. Factors Associated with PPI-Responders in Patients with Esophageal Eosinophilia

The results of multivariate analysis are shown in Table 3. After adjusting for presence of food allergy, indications for endoscopy, dysphagia, rings, and reflux esophagitis, GI screening, and the presence of reflux esophagitis were significantly associated with an increased OR for PPI-responders, and presence of rings was associated with a decreased OR for PPI-responders. Other factors were not significantly associated with PPI-responders in these patients with symptomatic esophageal eosinophilia. The positive predictive value for reflux esophagitis on endoscopy vis-a-vis the diagnosis

of PPI-responders was 17/18 (94.4 %) and the negative predictive value was 17/41 (41.5%). The positive predictive value for the absence of rings on endoscopy vis-a-vis the diagnosis of PPI-responders was 29/36 (80.1 %) and the negative predictive value was 11/23 (47.8%).

Table 1. Clinical characteristics of PPI-responders and non-responders.

	PPI-Responders (*n* = 41)	**Non-Responders (*n* = 18)**	***p***
Demographics (*n*, % or median, IQR)			
Age (years)	46 (26–73)	44 (28–72)	0.779
Male sex	27 (65.9)	12 (66.7)	1
Body mass index (kg/m^2)	22.7 (17.9–37.2)	23.8 (18.7–39.1)	0.242
Smoking habits	14 (34.1)	5 (27.8)	0.766
Drinking habits	27 (67.5)	11 (61.1)	0.767
Duration of illness (months)	9 (1–240)	12 (1–60)	0.844
Indications of endoscopy GI screening/further examination for upper GI symptoms	28 (68.3)/13 (31.7)	6 (33.3)/12 (66.7)	0.021
Allergy (*n*, %)			
Any	28 (68.3)	14 (77.8)	0.545
Asthma	7 (17.1)	4 (22.2)	0.721
Rhinitis	21 (51.2)	6 (33.3)	0.262
Dermatitis	4 (9.8)	3 (16.7)	0.664
Food	7 (17.1)	6 (33.3)	0.187
Blood test (*n*, % or median, IQR)			
WBC (/μL)	5600 (3500–9200)	5500 (4400–8400)	0.787
Eosinophil (/μL)	307.5 (100.8–1002.8)	380.8 (110–918.4)	0.615
Eosinophil (%)	5 (1.8–15.0)	5.9 (2.1–14.1)	0.551
IgE (IU/mL)	120 (5–2100)	230 (28–550)	0.176
Positive radioallergosorbent test	14 (40)	8 (53.3)	0.536
Helicobacter pylori infection	10 (24.4)	6 (33.3)	0.533
Symptoms (*n*, %)			
Dysphagia	29 (70.7)	16 (88.9)	0.189
Heartburn	12 (29.3)	3 (16.6)	0.354
Chest pain	6 (14.6)	4 (22.2)	0.475

Data are shown as medians with interquartile ranges or numbers with percentages. EoE: eosinophilic esophagitis; PPI: proton pump inhibitor; IQR: interquartile range; GI: gastrointestinal; WBC: white blood cell; IU: international unit.

Table 2. Endoscopic characteristics and histological feature of PPI-responders and non-responders before PPI therapy.

	PPI-Responders (*n* = 41)	**Non-Responders (*n* = 18)**	***p***
Endoscopic findings and histological feature (*n*, % or median, IQR)			
EREFS findings			
Linear furrows	37 (90.2)	17 (94.4)	1
grade 0	4	1	
grade 1	37	17	
Rings	12 (29.3)	11 (61.1)	0.041
grade 0	29	7	
grade 1	11	7	
grade 2	1	4	
grade 3	0	0	

Table 2. *Cont.*

	PPI-Responders (n = 41)	Non-Responders (n = 18)	p
White plaques	20 (48.8)	12 (66.7)	0.262
grade 0	21	6	
grade 1	15	8	
grade 2	5	4	
Edema	35 (85.7)	17 (94.4)	0.422
grade 0	6	1	
grade 1	35	17	
Strictures	0 (0)	0 (0)	
grade 0	41	18	
grade 1	0	0	
Reflux esophagitis	17 (41.5)	1 (5.6)	0.006
Los Angeles classification			
grade A	12	1	
grade B	4	0	
grade C	1	0	
grade D	0	0	
Hiatal hernia	6 (14.6)	4 (22.2)	0.475
Atrophic gastritis	11 (26.8)	4 (22.2)	1
Maximum eosinophil count (per hpf)	35 (15–110)	40 (20–170)	0.537

Data are shown as medians with interquartile ranges or numbers with percentages. EoE: eosinophilic esophagitis; PPI: proton pump inhibitor; IQR: interquartile range; EREFS: endoscopic reference score; hpf: high-power field.

Table 3. Risk factors of PPI-responders.

	Multivariate Analysis		
	OR	95% CI	p
Food allergy	0.223	0.029–1.690	0.146
GI screening	6.740	1.200–37.900	0.030
Dysphagia	0.145	0.011–1.890	0.141
Presence of rings	0.081	0.029–0.501	0.007
Presence of reflux esophagitis	9.490	1.010–89.100	0.049

Data were adjusted for food allergy, indications for endoscopy, dysphagia, rings, and reflux esophagitis. PPI: proton pump inhibitor, GI: gastrointestinal, OR: odds ratio, CI: confidence interval.

3. Discussion

In the present study, approximately 70% of patients with EoE responded to PPI therapy. PPI-responders and non-responders had similar clinical characteristics. However, multivariate analysis identified endoscopy for screening, reflux esophagitis, and the absence of rings as factors significantly associated with PPI-responders. These results suggest that such endoscopic findings, especially during GI screening, might predict the response to PPI therapy in Japanese patients with symptomatic esophageal eosinophilia.

A recent meta-analysis showed that PPI therapy led to a clinical response in 60.8% (95% CI, 43.4–72.2%) and histological remission in 50.5% (95% CI, 42.2–58.7%) of patients with symptomatic esophageal eosinophilia [16]. In the present study, a histological response as well as clinical improvement were seen in 41 (70%) out of 59 cases with symptomatic esophageal eosinophilia after PPI therapy. This somewhat higher rate of response to PPI might be due to the differences between populations and lesser severity of the disease, since no cases with strictures and food impaction were present in this study.

Several studies found no significant differences in the clinical characteristics between patients with PPI-responders and non-responders [15]. These results emphasize the fact that it is difficult to distinguish PPI-responders from non-responders based only on the clinical characteristics. However,

the rate of GI screening was significantly higher in PPI-responders than in non-responders in the present study. Therefore, the subjective symptoms in PPI-responders might be milder than in non-responders.

Several studies identified biomarkers for PPI-responders from non-responders or differences in the pathogenesis of these. Dellon et al. examined the expression of major basic protein (MBP), eotaxin-3, and tryptase in the esophageal mucosa by immunohistochemical staining [17]. They found significantly higher numbers of MBP-, eotaxin-3-, and tryptase- positive cells in patients with non-responders than in controls, but there were no differences between patients with PPI-responders and non-responders. Molina-Infante and colleagues evaluated expression of eotaxin-3 and Th-2 cytokine mRNA in esophageal biopsy specimens and found no significant differences in eotaxin-3, IL-5, and IL-13 mRNA expression levels between PPI-responders and non-responders [18]. In contrast, a transcriptome analysis by Wen et al. revealed that the expression of KCNJ2 (potassium inwardly-rectifying channel, subfamily J, member 2/Kir2.1) was significantly elevated in patients with non-responders compared to PPI-responders [19]. Our previous study showed that intraepithelial basophils infiltration was significantly higher in non-responders than in PPI-responders [20]. We did not assess histological findings such as intraepithelial basophil infiltration in this study, because it was not included in the diagnostic criteria of EoE. Considered together, these findings indicate that there might be some differences between PPI-responders and non-responders, although similar eosinophilic responses are ultimately achieved in both.

In the present study, the prevalence of reflux esophagitis was significantly higher in PPI-responders (41.5%) than in non-responders (5.6%). A recent Japanese study comparing 16 cases of PPI-responders and 11 cases of non-responders documented a more frequent presence of reflux esophagitis (18.8%) in PPI-responders than in non-responders (0%), although the difference was not statistically significant [13]. Savarino et al. analyzed 17 patients with PPI-responders and 35 patients with non-responders. They reported that PPI-responders had a higher prevalence of erosive esophagitis than non-responders (35% vs. 9%, $p = 0.04$) [21]. Therefore, the acid reflux might play a role in the pathogenesis of PPI-responders. Two mechanisms of response to PPI in patients with EoE have been proposed: acidity reduction and anti-inflammatory effects of PPIs [22]. Since the eosinophilic response is triggered by infiltration of an allergic antigen from the damaged mucosa [23], repair of the acid-induced esophageal epithelial injury by a PPI can prevent the penetration of the antigen. A response to PPI therapy was observed in 80% and 33%, respectively, in patients with pathological (abnormal acid exposure time) and normal esophageal acid exposure on esophageal pH monitoring [3]. On the other hand, Lucendo et al. reported in a meta-analysis that response to PPI therapy was observed in 65% and 49%, respectively, in patients with pathological and normal esophageal acid exposure on esophageal pH monitoring. However, no significant difference was found [16]. The higher prevalence of reflux esophagitis in PPI-responders could lead to a positive response to PPI therapy by the mechanisms described above. This is further supported by the fact that reflux esophagitis was relieved by PPI therapy in all PPI-responders.

Several typical endoscopic findings, including edema, decreased vascularity, linear furrows, white plaques, rings, and strictures, have been associated with PPI-responders and non-responders, but two prospective studies showed different results with respect to the prevalence of such endoscopic findings. Dellon et al. analyzed 65 cases, including 24 with PPI-responders and 41 with non-responders, and found that rings (63% versus 100%, $p = 0.001$), linear furrows (58% versus 92%, $p = 0.008$), narrowing (8% versus 33%, $p = 0.03$), and decreased vascularity (0% versus 1%, $p = 0.004$) were less likely to occur in PPI-responders [14]. In contrast, Molina-Infante et al. analyzed 53 cases, including 23 with PPI-responders and 30 with non-responders, and founded no differences in the prevalence of these endoscopic findings [18]. These discrepancies could be a result of heterogeneity of the study subjects and endoscopic diagnostic agreement. In the present study, the prevalence of rings in non-responders (61.1%) was significantly higher than that in PPI-responders (29.3%). Since the rings indicate chronic inflammation and remodeling of the esophagus caused by epithelial changes, angiogenesis, fibrosis,

and muscle hypertrophy [24–26], it is conceivable that the duration of the disease and the degree of inflammation might be related. However, the present study revealed no significant differences in the duration of the illness and the maximum esophageal eosinophil counts between PPI-responders and non-responders. Although it is not clear as to why the prevalence of rings was higher in non-responders than in PPI-responders, it is possible that PPI-responders had a milder disease than non-responders did, resulting in a difference in their responsiveness to PPI therapy. We don't know why rings revealed among milder diseases of EoE (PPI-responders), but it seems that there is some degree diversity of inflammation and fibrosis in PPI-responders.

It is known that approximately 50% of Japanese adults are infected with *Helicobacter pylori* [27, 28]. Although we investigated the prevalence of *Helicobacter pylori* infection and atrophic gastritis, no differences were found between PPI-responders and non-responders. The clinical data were collected before PPI treatment in patients with EoE and *Helicobacter pylori* eradication therapy were not performed during this study. We think it is difficult to discuss about association between *Helicobacter pylori*-positive EoE patients and PPI-treatment in EoE in this study.

This study has some limitations. First, it was conducted at a single center. Although we enrolled all the patients with EoE at our hospital, the sample size might be small. Moreover, the detection power was low because the patient numbers between the two groups were different. The prevalence of EoE is still low in Japan, and therefore a multicenter study will be needed to confirm our results. Second, we did not perform esophageal impedance pH monitoring. The nature of this reflux esophagitis, such as acid reflux esophagitis, alkaline reflux esophagitis, and the mixed acid-bile reflux esophagitis, were unknown in this study. Since the prevalence of reflux esophagitis was significantly higher in PPI-responders than in non-responders, a further study to examine the role of acid reflux in the pathogenesis of PPI-responders is necessary. Third, only two authors assessed the endoscopic images retrospectively in this study. There might be subjective differences in the interpretation of endoscopic diagnosis of EoE, especially in the assessment for rings, since transient, concentric mucosal rings [29]. However, all images were captured by at least one experienced endoscopist, suggesting a low possibility of misdiagnosis of the rings. Fourth, we treated patients with the Japanese standard dose of PPI, but ACG Guidelines recommend high-dose schedules. There is a possibility that the non-responders included some PPI-responders who responded to a high dose of PPI but did not respond to a standard dose of PPI. However, the standard dose of PPI was used for EoE in several Japanese studies [5,13].

In conclusion, this study suggests that the presence of reflux esophagitis and absence of rings during upper gastrointestinal endoscopy especially during GI screening might be factors predicting response to PPI therapy in Japanese patients with EoE.

4. Materials and Methods

4.1. Patients and Study Design

Seventy-eight adult patients who visited our hospital for esophageal eosinophilia defined as an intraepithelial eosinophilic infiltration of $\geq$15 per hpf were enrolled between April 2010 and April 2017 at our department of Osaka City University hospital, a tertiary referral hospital. The inclusion criterion was the presence of at least one esophageal symptom such as dysphagia, heartburn, and chest pain. Patients who had no esophageal symptoms, had received steroid inhalation therapy for bronchial asthma, or had received topical or systemic steroid therapy for EoE before PPI therapy were excluded. Cases with other causes of esophageal eosinophilia, including pemphigus, pemphigoid, and eosinophilic gastroenteritis, were also excluded. Enrolled patients were treated with a standard dose of PPIs (lansoprazole 30 mg, rabeprazole 10–20 mg, and esomeprazole 20 mg) for eight weeks, like several Japanese studies [5,13]. Clinical, endoscopic, and histological improvements were assessed after PPI therapy. We collected clinical data such as age, sex, body mass index (BMI), smoking and drinking habits, duration of illness, indications for endoscopy, allergic conditions and diseases,

symptoms, endoscopic findings, and results of blood tests, including peripheral eosinophil counts, serum IgE level, radioallergosorbent test results, and *Helicobacter pylori* antibody data from the medical records before administering PPI therapy. We defined the period from the appearance of the symptoms to the diagnosis as the duration of illness. Indications for endoscopy includes "GI screening" and "further examination for upper GI symptoms". GI screening means that subjects who visited for routine health check-ups were proven to be symptomatic by the interview after endoscopy. This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and the protocol was approved by the Ethical Committee of Osaka City University Medical School (no. 3591; 27 October 2016). All study subjects have given written informed consent.

4.2. Endoscopic Assessment and Biopsy Protocol

Signs of EoE, reflux esophagitis, hiatus hernia, and atrophic gastritis were assessed endoscopically before the PPI treatment. Endoscopic images were retrospectively reviewed and assessed by two authors independently. In cases where the findings did not match, we arrived at a consensus by mutual discussion. Two authors also assessed the endoscopic features after PPI therapy. We calculated the kappa coefficient to assess the inter-rater variability [30]. Endoscopic signs of EoE included (a) rings with a corrugated appearance or trachealization, (b) white plaques appearing as punctate white nodules, dispersant flocculant material, or in a granular pattern, (c) linear furrows representative of mucosal edema and thickening with vertical lines along the length of the esophageal mucosa, and (d) strictures [9,24]. We evaluated the signs of EoE according to the endoscopic reference score (EREFS) classification system [31]. Reflux esophagitis was diagnosed according to the Los Angeles classification [32]. Hiatus hernia was defined as an apparent separation of the esophagogastric junction and the diaphragmatic impression by more than 2 cm on endoscopy [33]. Atrophic gastritis was deemed present if atrophic gastritis had a severity of grade C-2 or higher [34]. After PPI therapy, we performed an upper gastrointestinal endoscopy, and the esophageal findings were re-evaluated. A total of 5–6 biopsy specimens were obtained from the esophagus: two from the distal esophagus, two from the middle esophagus, and 1–2 from the proximal esophagus. These biopsy samples were stained with hematoxylin-eosin, and intraepithelial eosinophils were counted on an optical microscope (Figure 2).

4.3. Definition of PPI Responsiveness

We evaluated the clinical symptoms and histological improvement after PPI therapy. A diagnosis of PPI-responders was considered if the subjective symptoms were relieved and the intraepithelial eosinophilic infiltration dropped to <15 per hpf on every biopsy after the PPI treatment, while EoE was diagnosed when an intraepithelial eosinophilic infiltration of $\geq$15 per hpf was seen on any of the biopsy samples.

4.4. Statistical Analysis

Data are shown as medians with interquartile ranges, and numbers and frequencies. Data were analyzed by Fisher's exact test and the Mann–Whitney *U* test for statistical comparison. After confirming a significant difference in each test, data were analyzed by logistic regression analysis. We selected GI screening, rings, and reflux esophagitis because these p values are less than 0.05 in univariate analysis. Although the p value is not less than 0.05, dysphagia is the most typical symptoms in EoE and food allergy is considered as the cause of EoE [2,14]. We selected them as confounding factors with p values less than 0.2. Although males are predominant in EoE, there were no differences between PPI responders and non-responders between males and females in univariate analysis as we showed in Table 1. (males: 65.9% in PPI-responders versus 66.7% in non-responders, $p = 1$). Therefore, we did not adjust the proportion between males and females in multivariate analysis in this study. The level of statistical significance was set to $p < 0.05$. All statistical analyses were performed using the statistical software EZR (Easy R, Saitama, Japan), which is based on R and R commander [35].

Author Contributions: Study concept and design: A.H., T.S., and Y.F.; acquisition of data: A.H., T.S., N.I., and Y.F.; analysis and interpretation of data: A.H., T.S., and Y.F.; drafting of manuscript: A.H.; critical revision of the manuscript for important intellectual content and approval of the final version: A.H., T.S., N.I., R.U., A.S., K.O., K.T., S.H., Y.N., F.T., N.K., H.Y., T.T., T.W., and Y.F.; supervision: Y.F.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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