Supplemental Methods

Protocol

This study was approved by the Institutional Review Board at Cincinnati Children's Hospital Medical Center (CCHMC). Inclusion criteria were that patients had consented to be entered into the Cincinnati Center for Eosinophilic Disorders (CCED) database and had an endoscopy with esophageal biopsies performed at CCHMC. Additional criteria were that slides were available for review after the surgical pathology report that specified an exact peak eosinophil count for the esophageal biopsies was issued. Biopsies meeting the specified criteria, obtained between June 2008 and November 2010 and reevaluated as part of the general quality assurance procedure for biopsy data entered into the CCED database, and between December 2003 and October 2011, and reevaluated as part of another reported study, which included EoE patients with esophageal eosinophilia unresponsive to PPI therapy (4), were examined by one trained research assistant, who generated a peak intraepithelial eosinophil count independent of and blinded to the count specified in the surgical pathology report.

Histologic evaluation

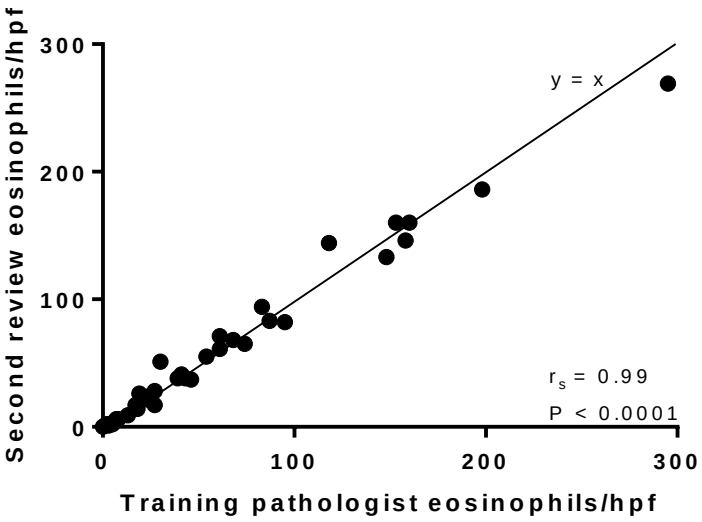
Hematoxylin- and eosin-stained esophageal sections were examined by light microscopy at 400X magnification. The initial peak counts were made by board-certified CCHMC pathologists (n = 9), and reported in the pathology report. All slides reviewed were from endoscopies performed at CCHMC; no slides included in the study were from an outside institution. A trained research assistant subsequently examined the same slides and recorded a peak eosinophil count at 400X magnification (second review). The research assistant was trained to examine every level of each biopsy present on a slide, identify the area with the greatest concentration of eosinophils, and count cells with nuclei and intensely red cytoplasmic granules to generate a peak eosinophil count. Granules without nuclei were not counted, and only intraepithelial eosinophils were counted; eosinophils in the lamina propria, papillae, and muscularis mucosa were excluded. The research assistant obtained peak eosinophil counts using one microscope with a 400X hpf area of 0.239 mm^2. In order to account for the variability in hpf size in the pathology

report counts, a stage micrometer was used to measure all the 400X hpfs in the pathology department at CCHMC that were used for the diagnostic slide review and the area of the hpfs ranged from 0.212 to 0.307 mm^2; the average area was 0.257 ± 0.037 mm^2.

To ensure the validity of the peak eosinophil count obtained by the research assistant, the training pathologist reevaluated 40 biopsies using a microscope with a hpf area of 0.3 mm^2. In this comparison, the correlation between the count obtained by the research assistant and the count obtained by the training pathologist was very strong (r_s = 0.99, $P < 0.0001$) (Supplemental Figure 1) with no significant median difference (P = 0.10).

Statistical analysis

As the sample sets did not conform to a normal distribution, the Spearman coefficient of correlation and the Wilcoxon matched-pairs signed-rank test were used to compare the pathology report counts to the second review counts and the training pathologist's counts to the second review counts. The mean, median, and standard deviation (SD) were determined for reported ranges. Additional statistical analyses were performed using GraphPad Prism version 5.00 for Windows and GraphPad Software, San Diego, CA, USA, www.graphpad.com.



Supplemental Figure 1. Correlation of training pathologist's eosinophil counts and second review counts. Spearman correlation coefficient (r_s) was calculated to compare the counts (n = 40). Line y = x represents where the values would be located if the training pathologist's count and second review count were equivalent. hpf, high-power field.

Supplemental Table 1. Positive Predictive Value, Negative Predictive Value, sensitivity, and specificity for pathology reports. PPV, positive predictive value; NPV, negative predictive value.

		Second Review		
		EoE	**no EoE**	**Total**
Pathology report	**≥15 eosinophils/hpf**	**96**	**1**	**97**
	<15 eosinophils/hpf	**24**	**356**	**380**
	Total	**120**	**357**	**477**

Sensitivity = 80%	PPV = 99%
Specificity = >99%	NPV = 94%