APPENDIX

SUPPLEMENTAL TABLE 1. Diagnostic performance of volumetric laser endomicroscopy scoring index (OCT-SI) by dysplasia score threshold

VLE, dysplasia index	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Diagnostic accuracy (95% CI)
≥1	100 (87-100)	2 (0-27)	90 (68-94)	33 (2-41)	69 (68-70)
≥2	93 (78-98)	18 (7-44)	71 (55-83)	48 (19-83)	69 (62-84)
≥3	70 (52-84)	60 (36-79)	80 (62-90)	47 (26-70)	67 (58-78)
4	14 (5-31)	93 (68-99)	80 (35-97)	34 (21-50)	40 (38-48)

OCT-SI, Optical coherence tomography scoring index; *VLE,* volumetric laser endomicroscopy; *CI,* confidence interval; *PPV,* positive predictive value; *NPV,* negative predictive value.

SUPPLEMENTAL TABLE 2. Diagnostic performance of pCLE and VLE by using the OCT-SI and VLE-DA*

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Diagnostic accuracy (95% CI)
pCLE	71 (55-83)	90 (54-98)	97 (81-100)	47 (24-68)	75 (66-86)
OCT-SI (score ≥3)	68 (52-81)	70 (38-89)	91 (74-97)	35 (16-59)	69 (66-70)
VLE-DA	77 (60-88)	93 (58-99)	98 (83-100)	50 (27-73)	80 (78-84)

pCLE, Probe confocal laser endomicroscopy; *VLE,* volumetric laser endomicroscopy; *OCT-SI,* optical coherence tomography scoring index; *VLE-DA,* VLE diagnostic algorithm.
*Endoscopic mucosal resection specimens containing low-grade dysplasia categorized as neoplastic.

SUPPLEMENTAL TABLE 3. Diagnostic performance of pCLE and VLE by using the OCT-SI and VLE-DA in patients not previously treated with radiofrequency ablation

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Diagnostic accuracy (95% CI)
pCLE	78 (47-82)	82 (31-78)	91 (58-90)	67 (20-66)	80 (66-100)
OCT-SI (score ≥3)	66 (47-82)	56 (31-78)	78 (58-90)	41 (20-66)	62 (31-92)
VLE-DA	86 (67-95)	87 (56-98)	94 (75-99)	74 (45-91)	87 (85-92)

pCLE, Probe confocal laser endomicroscopy; *VLE,* volumetric laser endomicroscopy; *OCT-SI,* optical coherence tomography scoring index; *VLE-DA,* VLE diagnostic algorithm.

VOLUMETRIC LASER ENDOMICROSCOPY DIAGNOSTIC ALGORITHM DEVELOPMENT

Training set

A training set of 30 EMR volumetric laser endomicroscopy (VLE) scans (nonneoplastic N = 2: nondysplastic Barrett's esophagus [BE] N = 6; low-grade dysplasia [LGD] N = 6; neoplastic N = 18: high-grade dysplasia [HGD] N = 10, intramucosal adenocarcinoma [IMC] N = 8) was examined by 2 investigators (C.L.L., E.C.G.) for features associated with dysplasia.

Partial effacement of mucosal layer

Squamous epithelium is characterized by a highly reflecting mucosal layer that sharply differentiates mucosa from submucosa. The observed mucosal layer was noted to be partially effaced in nonneoplastic BE, often containing several breaks and providing less distinction between mucosa and submucosa. Neoplastic scans more commonly had complete effacement or absence of the mucosal layer.

We performed systematic measurement of the longitudinal length of the partially effaced mucosal layer in the training set. We defined partial effacement of the VLE mucosal layer as a mucosal layer ≥2 mm (mean length of mucosal layer measured) in transverse cross-sectional length present in ≥50% of the scan. Complete effacement of the mucosal layer was defined by absence of a mucosal layer or its presence in <2 mm in transverse cross-section over <50% of the scan.

Nonneoplastic scans were more likely to contain a partially effaced mucosal layer (nonneoplastic, N = 11 [37%]; neoplastic, N = 5 [17%]; $P < .001$), whereas neoplastic scans were more likely to contain complete effacement of this layer (neoplastic, N = 13 [43%]; nonneoplastic, N = 1[3%]; $P < .001$). The mean (± standard deviation [SD]) length of the mucosal layer in nonneoplastic scans was 2.4 (1.7) mm, compared with 1.3 (1.0) mm in neoplastic scans ($P = .03$) over the length of the EMR specimen. The mean (± SD) EMR specimen length in the training set was 11 (± 8) mm, with no statistical difference between neoplastic and nonneoplastic EMR specimens ($P = .38$).

Atypical glandular structures

The presence of atypical glands is associated with BE dysplasia by using optical coherence tomography

(OCT).[10] With VLE, we hypothesized that there is a correlation between the number of atypical glands and dysplasia. To this end, we performed systematic measurements of gland cross-sectional surface areas and quantification of number of glandular structures in the training set. Glands were characterized as *normal appearing* if they showed a round and smooth contour with minimal to absent irregularity and as *atypical* if they showed an irregular shape, size, and distribution. Results from the training set analysis showed that a greater number of neoplastic scans contained >5 atypical glands per EMR specimen compared with nonneoplastic scans (neoplastic, N = 11 [33%]; nonneoplastic N = 2 [7%]; $P = .01$). The mean surface occupied by glandular structures was significantly higher for neoplastic EMR specimens compared with nonneoplastic EMR specimens (18.3 mm^2 vs 3.6 mm^2; $P < .001$). Scans with partial effacement of the mucosal layer (N = 16), having >5 atypical glands were significantly associated with the presence of neoplasia (χ^2 11.4; $P < .001$).

Surface and/or subsurface intensity ratios

In addition to the number of atypical glands and effacement of the mucosal layer, we investigated a previously developed OCT criterion that compares surface with subsurface intensity as a metric of surface maturation in BE.[5] Surface-to-subsurface intensity was measured across the entire VLE scan for each training set EMR specimen. An overall rating was determined based on the most prevalent ratio found in ≥50% of the scan (surface > subsurface intensity vs surface ≤ subsurface intensity). Among scans with complete effacement of the mucosal layer (N = 14), a ratio of surface intensity greater than subsurface intensity was significantly associated with the presence of neoplasia ($P < .001$) even in the absence of atypical glands.