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Outcome of endoscopic mucosal resection in Barrett's esophagus determined by systematic quantification of epithelial glands using volumetric laser endomicroscopy

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Abstract

Background: Dysplastic Barrett's esophagus (BE) lesions ≤2 cm in size can be targeted for en-bloc endoscopic mucosal resection (EMR). White-light endoscopy can underestimate the size of a lesion, limiting complete resection. Volumetric laser endomicroscopy (VLE) provides high-resolution cross-sectional imaging of BE. Epithelial glands are a VLE feature associated with BE dysplasia. We study the association between VLE gland quantification and outcome of resection.

Methods: EMR specimens of BE lesions targeted for en-bloc resection were imaged with VLE using an established protocol. Manual and automated quantification of epithelial glands was performed blinded to resection outcome. The presence of epithelial glands at the resection margins was recorded. Histologic en-bloc (R0) resection of the targeted lesion was defined by the absence and incomplete (R1) resection by the presence of dysplasia/neoplasia at specimen margins.

Results: Thirty-seven EMRs with a mean (standard deviation) size of 1.04 (0.37) cm were imaged with VLE. The highest grade of dysplasia found was low-grade dysplasia (n = 12), high-grade dysplasia (n = 19), and intramucosal cancer (n = 6). The en-bloc resection rate was 37.8% (R0, n = 14; R1, n = 23). The mean (standard deviation) number of epithelial glands quantified with VLE was 13.0 (6.7) and 28.8 (23.9) for R0 and R1 specimens, respectively, with a significant mean difference of 15.8 glands (95% confidence interval, 2-29; $P = .02$). The presence of glands at the specimen margin was associated with incomplete resection ($P < .001$).

Conclusion: Systematic quantification of BE epithelial glands using VLE can determine the outcome of endoscopic resection. VLE may have a potential role in assessment of lesion margins. (Gastrointest Endosc 2019;89:701-8.)

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INTRODUCTION

Barrett's esophagus (BE) is a premalignant condition that arises when normal stratified squamous epithelium is replaced by metaplastic columnar epithelium in the setting of chronic gastroesophageal reflux disease.[1] Endoscopic surveillance is recommended in patients with BE as a strategy to diagnose and treat dysplasia and early neoplasia before its progression to invasive cancer.[1] Endoscopic therapy consists of a combination of resection and ablative techniques. Endoscopic mucosal resection (EMR) is performed over areas of mucosal irregularity for histologic diagnosis and staging of the lesion.[2,3] When an endoscopically visible lesion is less than 2 cm in size, EMR can potentially offer a curative intervention by removing the lesion en-bloc.[4] Larger lesions can be removed in piecemeal fashion using EMR but this technique compromises adequate lateral margin assessment and the ability to determine the rate of curative resection. Alternative resection techniques such as endoscopic submucosal dissection (ESD) can be used to achieve en-bloc resection of larger lesions but may be associated with a higher rate of esophageal strictures, bleeding, and perforation.[5]

An accurate estimate of the lesion size is important in determining the appropriate endoscopic resection approach. The rate of margin-free resection of lesions with high-grade intraepithelial neoplasia or early adenocarcinoma using EMR is estimated to be 49.3%, suggesting that high-definition white-light endoscopy may underestimate BE lesion size.[6] Advanced imaging modalities such as volumetric laser endomicroscopy (VLE) may allow for a more precise estimate of lesion size because this technology is capable of wide-field, cross-sectional imaging across 6 cm of the esophagus at a depth of 3 mm with a lateral resolution of 7 μm (NvisionVLE Imaging System. NinePoint Medical, Bedford, Mass). VLE has previously been shown to accurately detect BE-associated dysplasia and can be used in conjunction with high-definition white-light endoscopy and narrow-band imaging to identify a lesion of interest.[7,8]

The role of VLE in guiding endoscopic therapy has not been studied previously. Salient VLE features associated with BE dysplasia include the morphology and number of epithelial glands, lack of layering, and higher surface signal intensity.[9] Atypical glands are characterized by their irregular shape and larger size, often containing intraluminal debris. We have previously demonstrated that the presence of more than 5 atypical glands is significantly associated with BE dysplasia.[7]

In this study, we hypothesized that systematic analysis of BE glands using VLE can help determine the outcome of resection. To this end, we performed manual and automated quantification of epithelial glands in EMR specimens targeted for en-bloc resection and imaged with VLE.

METHODS

Patient selection and clinical data

Patients with a history of dysplastic BE who underwent a clinically indicated EMR were retrospectively evaluated for inclusion in this study. Patients undergoing endoscopic

resection on BE lesions estimated to be ≤2.0 cm in size under endoscopic evaluation and with intent at en-bloc resection were included. Resection of lesions >2.0 cm in size or those performed in piecemeal fashion were excluded from the study.

Demographic information, including patient age and gender at time of EMR, was abstracted from medical records. Endoscopic records were reviewed for length of BE segment (centimeters), size of resected BE lesion (centimeters), and history of previous endoscopic therapy, including radiofrequency ablation, cryoablation, and EMR.

The primary aim of this study was to determine the association between epithelial gland count on VLE and the histologic outcome of resection. A secondary aim was to determine the association between epithelial glands involving the lateral margin(s) of the EMR specimen and the outcome of resection. This study was approved by the Institutional Review Board at our institution.

Imaging of EMR specimens with VLE

BE lesions were inspected with high-resolution white-light endoscopy and narrow-band imaging before resection. After endoscopic resection, EMR specimens were rinsed with a phosphate-buffered saline solution, oriented along their longitudinal axis, and placed inside a specially designed specimen holder for imaging with the Nvision VLE Imaging System (Fig. 1).[7] Cross-sectional and longitudinal images were obtained over the entire length of the EMR specimen. A total of 2 full VLE scans were performed per specimen. VLE scans were reviewed for imaging quality, including appropriate imaging probe centering, low number of imaging artifacts, and high signal-to-noise ratio. Only high-quality scans were selected for this study.

Histologic review of EMR specimens

After VLE imaging, the EMR specimens were sent for standard processing and histologic analysis, including highest grade of dysplasia (low-grade dysplasia [LGD], high-grade dysplasia [HGD], intramucosal cancer [IMC]) as well as lateral and deep margin involvement. En-bloc (R0) resection of the targeted lesion was defined by the absence of dysplasia and/or neoplasia at the specimen margins. Incomplete resection (R1) of the targeted lesion was defined by the presence of dysplasia and/or neoplasia at the specimen margins.

Manual epithelial gland quantification using VLE

All VLE scans of EMR specimens were reviewed in a blinded fashion by 2 users with experience in VLE image interpretation (C.L.L., A.K.K.) to assess the number and distribution of epithelial glands. Epithelial glands were manually quantified by reviewing each independent VLE frame in cross-section across the entire EMR specimen. Epithelial glands in a cluster configuration contain multiple small glands that can be difficult to quantify. When reviewing glands in a cluster configuration, reviewers considered 2 glands to be independent of each other if lamina propia was present between them. In order to directly compare manual with automated epithelial gland quantification, the manual epithelial gland quantification included both atypical and normal epithelial glands. VLE scans were also

reviewed for the absence or presence of epithelial glands at the EMR lateral margins. Epithelial glands were considered to involve the resection margins if found ≤0.5 mm from the specimen lateral margin. Submucosal cystic structures were not quantified as part of this analysis because these structures represent normal histologic structures (eg, dilated ducts of submucosal esophageal glands, submucosal vasculature) not directly associated with BE dysplasia.[10]

Automated computer-aided epithelial gland quantification using VLE

Automated image visualization enhancement (IVE) software (NinePoint Medical, Bedford, Mass) was used to visualize epithelial glands for quantification.[11] The IVE software renders an en-face view of the VLE scan with a superimposed topographic map of the surface area and distribution of epithelial glands. This is achieved by a series of image processing steps, including thresholding and delineation of hyporeflective structures (glands) on VLE cross-sectional view. The algorithm further distinguishes between epithelial glands and submucosal cystic structures when a layered architecture is present. IVE processed scans were used to calculate the surface area occupied by epithelial glands using the image processing software ImageJ 1.51n[12] (National Institutes of Health, USA). The outline of the en-face EMR was manually traced to avoid measurement of surrounding structures (eg, cork, fluid, etc.). The total surface area occupied by glands (displayed in white) was recorded for each EMR specimen (Fig. 2). Epithelial glands were defined as involving the resection margin if found ≤0.5 mm from the en-face EMR outline. The IVE software was designed for analysis of second-generation VLE scans.

Statistical analyses

Baseline characteristics and outcomes were analyzed using the mean, standard deviation (SD), and confidence intervals (CI) for continuous variables and the percentage for nominal variables. The characteristics of R0 lesions were compared with R1 using the Student t test for continuous predictors and the Fisher exact test for nominal predictors. We compared the manual gland count and the degree of dysplasia using one-way analysis of variance. In addition, we evaluated the correlation between manual quantification and automated computer-aided epithelial gland quantification using Pearson correlation. Two-sided P values less than .05 were considered statistically significant. All analyses were performed using JMP software (Version 10, SAS Inc Cary NC).

RESULTS

A total of 307 EMR specimens were imaged using VLE between January 1, 2012, and December 31, 2017. Of these, 265 EMRs were performed in piecemeal fashion or on lesions >2.0 cm in size and excluded from the study. The remaining 42 EMRs were performed with intent at endoscopic en-bloc resection using a cap and snare technique (Olympus USA, Center Valley, Pa). BE lesions were described as nodular and/or having an irregular mucosal pattern (Paris Classification type IIa or IIb) under high-definition white-light endoscopy and narrow-band imaging. Endoscopic characteristics were similar across all lesions targeted for en-bloc resection. Five EMRs had poor VLE imaging quality and were excluded. The final study dataset consisted of 37 EMR specimens obtained from 31 patients, of whom 28 (90%)

were male. Four patients underwent multiple EMRs as follows: 1 patient underwent EMR of 2 different lesions at the same session, 1 patient underwent EMR of 2 distinct lesions in 2 separate sessions, 1 patient underwent EMR of 3 distinct lesions in 3 separate sessions, and 1 patient underwent EMR of 3 distinct lesions in 2 separate sessions. Fifteen EMR specimens were imaged using the first-generation Nvision VLE Imaging System, and 22 were imaged using the second-generation system. The study design is illustrated in Figure 3.

The mean (SD) age of the patients at the time of study inclusion was 67.5 (10.8) years. The mean (SD) length of the BE segment was 5.46 (4.14) cm; 19 (51.4%) patients had short-segment BE and 18 (48.6%) had long-segment BE. The mean (SD) size of EMR specimens was 1.04 (0.37) cm. The highest grade of dysplasia in the EMR specimens was LGD (n = 12), HGD (n = 19), and IMC (n = 6). A total of 24 (65%) patients underwent previous endoscopic therapy; 17 (46%) patients had received ablation (radiofrequency ablation or cryoablation) and 19 (51%) patients had undergone prior EMR. Twelve (32%) patients had undergone both ablation and EMR procedures previously.

Histology of EMR specimens

Histologic en-bloc resection (R0) was achieved in 14 (37.8%) and incomplete resection (R1) in 23 (62.2%) lesions. The histology of R0 specimens included LGD (n = 5), HGD (n = 7), and IMC (n = 2). The histology of R1 specimens included LGD (n = 7), HGD (n = 12), and IMC (n = 4). There was no statistical difference between resection outcome (R0 vs R1) and grade of dysplasia ($P = .56$). There was also no statistical difference between resection outcome (R0 vs R1) and a previous history of endoscopic therapy ($P = .44$).

Manual epithelial gland quantification

On manual quantification, the mean (SD) number of epithelial glands per EMR specimen was 22.8 (20.7) with a mean (SD) number of glands per VLE frame of 0.11 (0.10). The mean (SD, 95% CI) number of glands was 13.0 (6.7; 95% CI, 9.11-16.89) and 28.8 (23.9; 95% CI, 18.49-39.16) for R0 and R1 specimens, respectively, with a statistically significant mean difference of 15.8 glands ($P = .02$) between the 2 groups (Table 1, Fig. 4). The mean (SD) number of glands per VLE frame was 0.06 (0.03) in R0 specimens and 0.14 (0.11) in R1 specimens ($P = .02$). In our dataset, a gland count ≥28 glands predicted incomplete resection (R1) with 100% positive predictive value and 100% specificity (area under the receiver operating characteristic curve = 0.70). The epithelial gland count was significantly associated with EMR size ($P = .03$) with an increase of 3.4 epithelial glands for every 1 mm increase in EMR size (Supplementary Fig. 1, available online at www.giejournal.org).

The mean (SD) manual epithelial gland count was 20.3 (16.6), 21.8 (19.2), and 31.2 (32) in specimens with LGD, HGD, and IMC, respectively. The mean (SD) number of glands per VLE frame was 0.10 (0.0), 0.11 (0.11), and 0.13 (0.12) in specimens with LGD, HGD, and IMC, respectively. There was no significant difference in epithelial manual gland count or number of glands per VLE frame and grade of dysplasia ($P = .56$ and $P = .85$, respectively). We found no statistical difference between manual gland quantification and a previous history of endoscopic therapy ($P = .81$).

Computer-aided epithelial gland surface area quantification

IVE software quantification of epithelial glands was performed on 20 of 22 EMRs imaged using the second-generation Nvision VLE Imaging System; IVE analysis was not performed on 2 EMRs scans due to image rotational motion but this did not preclude manual quantification. This dataset included 6 (30%) R0 and 14 (70%) R1 specimens. Histology showed LGD (n = 2), HGD (n = 3), and IMC (n = 1) in R0 specimens and LGD (n = 3), HGD (n = 7), and IMC (n = 4) in R1 specimens. There was no statistical difference between resection outcome (R0 vs R1) and grade of dysplasia ($P > .99$).

The mean (SD) surface area occupied by epithelial glands in R0 and R1 specimens was 0.98 (1.1) mm^2 and 2.3 (2.4) mm^2, respectively. There was a strong correlation between manual gland count and IVE software epithelial gland surface area (R^2, 0.81, $P < .001$) (Fig. 5). In this limited sample size, statistical significance was not reached between IVE gland surface area and resection outcome ($P = .26$).

Analysis of epithelial glands involving the EMR margin

On cross-sectional VLE review, epithelial glands were found to involve the resection margin in 25 (67.6%) specimens, of which 5 (20.0%) were considered R0 and 20 (80.0%) were R1 resections. The resection margins of 12 (32.4%) specimens were free of glands, of which 9 (75%) were R0 and 3 (25%) were R1 resections. A significant difference between the status of the EMR margin (presence vs absence of glands) and the resection outcome (R0 vs R1) was observed ($P < .01$). A previous history of endoscopic therapy did not appear to have a significant impact on this outcome ($P = .56$).

Automated analysis showed that epithelial glands involved the en-face EMR margin outline in 12 (60.0%) specimens, of which 1 (8.0%) was an R0 specimen and 11 (92.0%) were R1 specimens. Eight (40.0%) specimens were free of glands at the en-face EMR margins, of which 5 (62.5%) were R0 specimens and 3 (37.5%) were R1 specimens. A significant difference between the status of the en-face EMR margin (presence vs absence of glands) and the resection outcome (R0 vs R1) was observed ($P = .01$).

DISCUSSION

An accurate estimation of the size of a BE lesion is important in deciding the appropriate endoscopic resection approach. High-definition white-light endoscopy may underestimate lesion size and in turn, lead to a noncurative resection. VLE is an advanced imaging technology that can evaluate a BE lesion in cross-section and at microscopic resolution. In this study, we explore whether the VLE feature of epithelial glands can be used to determine the outcome of endoscopic resection of a BE lesion.

The histologic en-bloc resection rate reported in this study was 37.8% and is similar to previous literature.[13] We demonstrate that en-bloc resection specimens (R0) contain a significantly fewer number of epithelial glands compared with noncurative resection specimens (R1) (13.0 vs 28.8). Given that the size of the EMR specimen may have an impact on epithelial gland quantification, we calculated the number of glands per VLE frame

with similar results. We also highlight that previous endoscopic therapy with either ablation or EMR does not have an impact on these results.

IVE software was used to delineate hyporeflective glandular structures and display the information in an en-face view. A limitation of this approach is that the IVE software does not account for gland size or morphology. Consequently, larger glands that occupy more surface area may be associated with a lower manual gland count. We validated the use of this software by comparing epithelial gland manual quantification to surface area (mm^2) and found a strong correlation between these metrics. Because the IVE software was designed for the second-generation Nvision VLE system, we were unable to apply this approach to our entire dataset. No statistical correlation was found between epithelial surface area measured using the IVE software and outcome of resection (R0 vs R1) likely due to the limited sample size.

A secondary aim of this study was to determine the association between epithelial glands involving the specimen lateral margins and the outcome of resection. We found that the presence of epithelial glands at the resection margins was significantly associated with an incomplete resection in both manual and IVE software analysis. Epithelial glands were considered to involve the lateral margin if present within 0.5 mm of the resection margin as a counterpart to the histologic definition of margin involvement. Determining the presence of epithelial glands directly at the specimen margin can be challenging due to image artifacts (liquid, bubbles, cautery) at the air-tissue interface. However, this limitation is unique to ex vivo VLE analysis and should not affect in vivo interpretation of margin assessment.

Taken together, our results suggest the potential role for VLE in providing guidance at the time of endoscopic resection. In a clinical setting, piecemeal EMR or ESD could be considered for a BE lesion imaged with VLE that is found to have a large number of epithelial glands or a large surface area occupied by glands as determined by the IVE software. VLE with laser marking could then be used to outline the margins of the lesion by defining a region devoid of epithelial glands. Although manual quantification of glands may not be clinically practical in an in vivo setting due to time constraints, we foresee that automated quantification using IVE software would be more useful in this setting. In this process, it would also be important to verify the presence of atypical glands on cross-sectional imaging given the association of this feature with dysplasia.

Although systematic gland quantification was performed in endoscopic resection specimens using a validated VLE imaging protocol, we are unable to correlate our findings with in vivo VLE scans to determine the true size of the BE lesion, in particular for R1 resection specimens. A prospective in vivo study that uses VLE to estimate BE lesion size before endoscopic resection is warranted to validate the findings of this study. This approach could also help determine if VLE-guided endoscopic therapy has an impact on clinical outcomes.

The role of VLE in endoscopic therapy of BE is being defined. This is the first study to suggest that systematic quantification of a VLE feature can be used to estimate the outcome of endoscopic resection. Systematic epithelial gland quantification may be an important step in determining BE lesion size and an approach to endoscopic resection using VLE.

Furthermore, computer-aided quantification tools, such as IVE, may be applied to guide the endoscopist in estimating lesion size and resectability. The findings in this study need to be corroborated with in vivo analysis of epithelial glands in order to establish the use of VLE in BE endoscopic therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

BE	Barrett's esophagus
CI	confidence interval
EMR	endoscopic mucosal resection
ESD	endoscopic submucosal dissection
HGD	high-grade dysplasia
IMC	intra-mucosal adenocarcinoma
IVE	image visualization enhancement
LGD	low-grade dysplasia
SD	standard deviation
VLE	volumetric laser endomicroscopy

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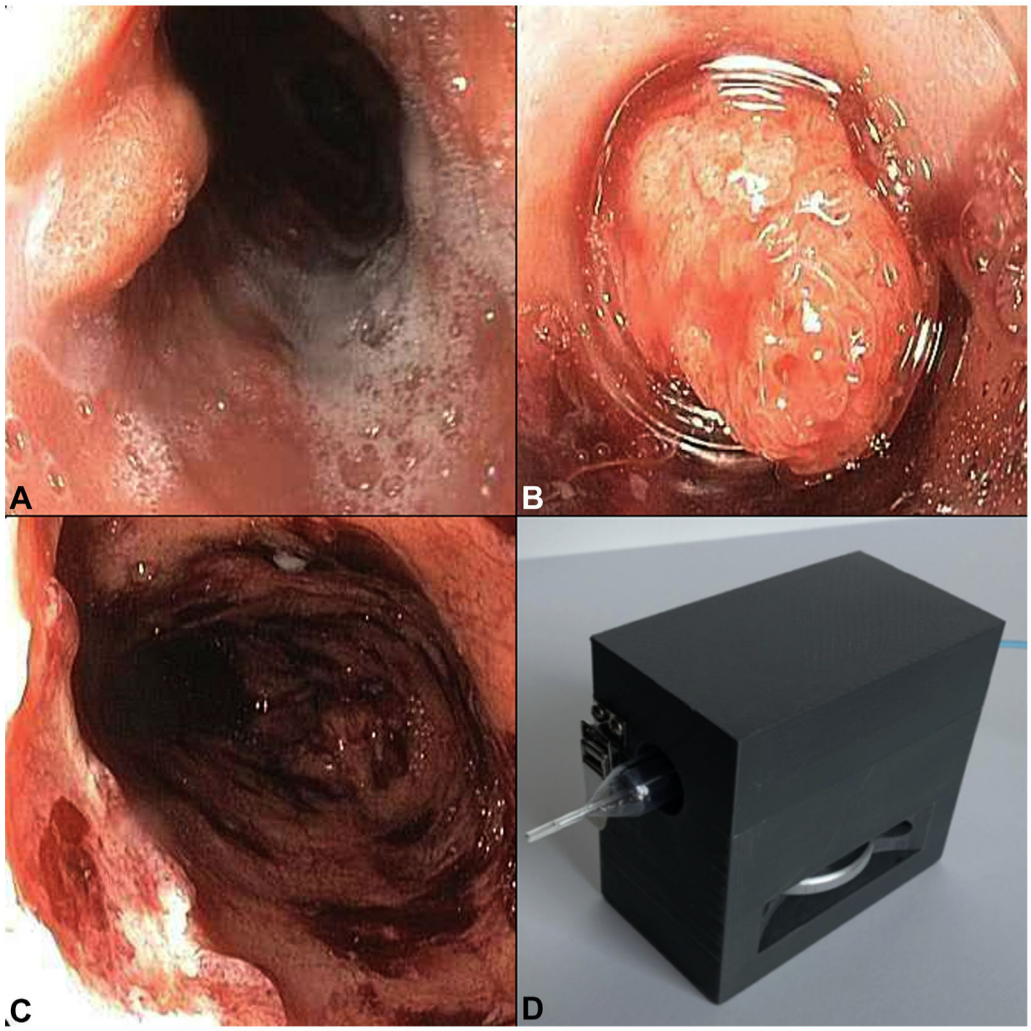

Figure 1.

A, Segment of Barrett's esophagus (BE) with a nodular lesion (Paris classification type IIa) under high-definition white-light endoscopy. **B**, En-bloc EMR of the BE lesion using a cap-snare technique. **C**, Post-EMR site showing no residual nodularity. **D**, Ex vivo volumetric laser endomicroscopy imaging of the EMR specimen within a specimen holder.

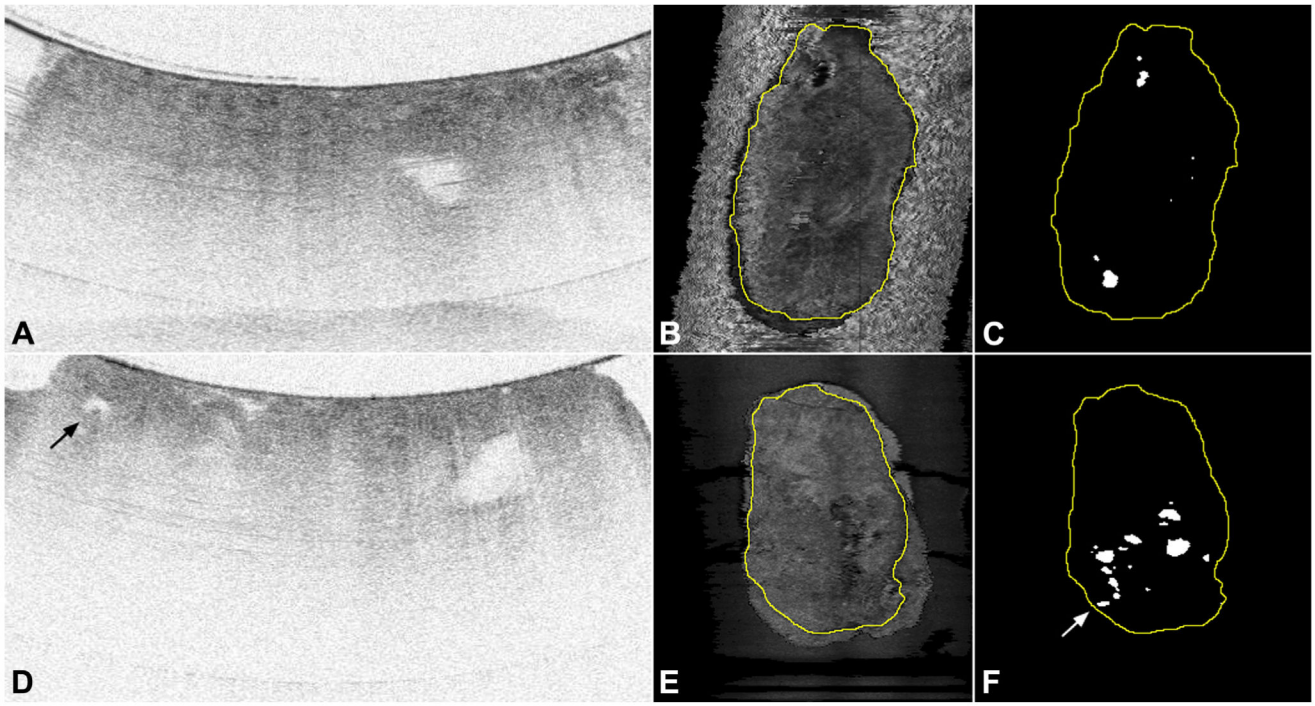

Figure 2.

A and D, Volumetric laser endomicroscopy (VLE) scan of EMR specimens. The VLE scan is rendered to generate an en-face view of the specimen (**B and E**). The outline of the EMR (yellow) is selected and superimposed over a topographic map (**C and F**) of epithelial glands (displayed in white) generated using image visualization enhancement (IVE) software. An epithelial gland count is performed by review of each individual frame across the EMR in VLE cross-section. The total surface area (mm^2) occupied by glands is quantified using the IVE en-face image. A VLE scan of an en-bloc resection specimen (R0) with a total cross-sectional gland count of 9 and a gland surface area of 0.69 mm^2 with glands >0.5 mm from the specimen margin is shown in (**C**). A VLE scan of an incomplete resection specimen (R1) with a total cross-sectional gland count of 28 and a gland surface area of 2.0 mm^2 with glands involving the specimen margin (*arrow*) at <0.5 mm is shown in (**F**).

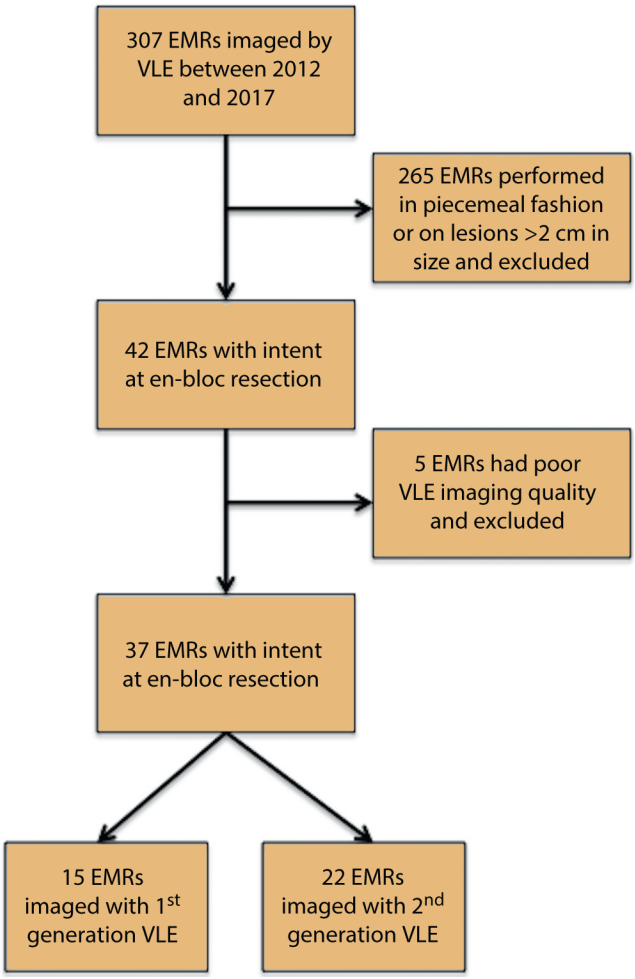

Figure 3.

Flow diagram outlining the study design and inclusion and exclusion criteria. *VLE*, Volumetric laser endomicroscopy.

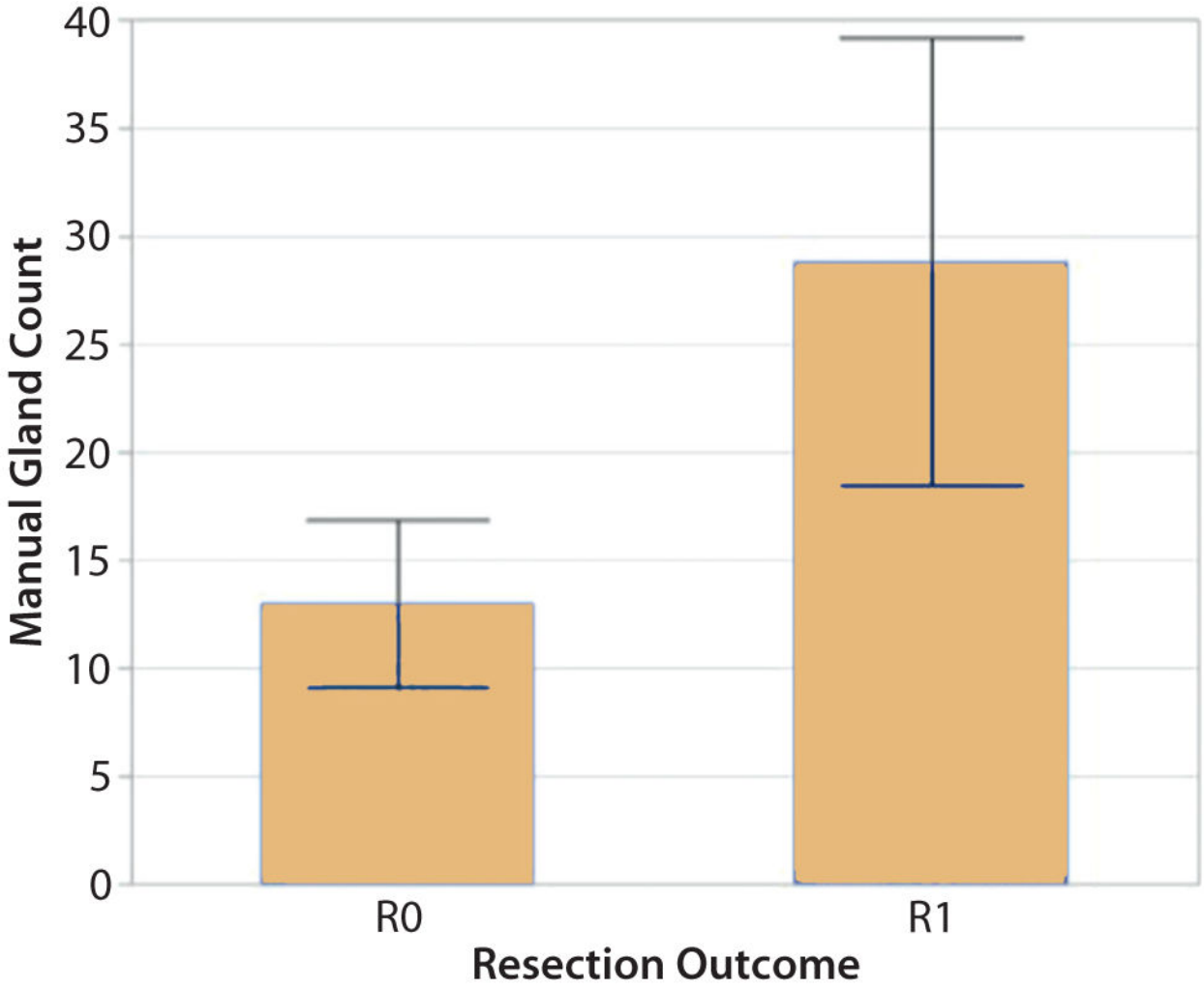

Figure 4.

The distribution of manual gland counts in EMR specimens with en-bloc (R0) and incomplete (R1) resection. The mean (95% confidence interval) number of glands was 13.0 (9.11-16.89) and 28.8 (18.49-39.16) for R0 and R1 specimens, respectively, with a statistically significant mean difference between groups of 15.8 glands ($P = .02$). The bars represent the mean number of glands and the error bars represent the 95% confidence interval.

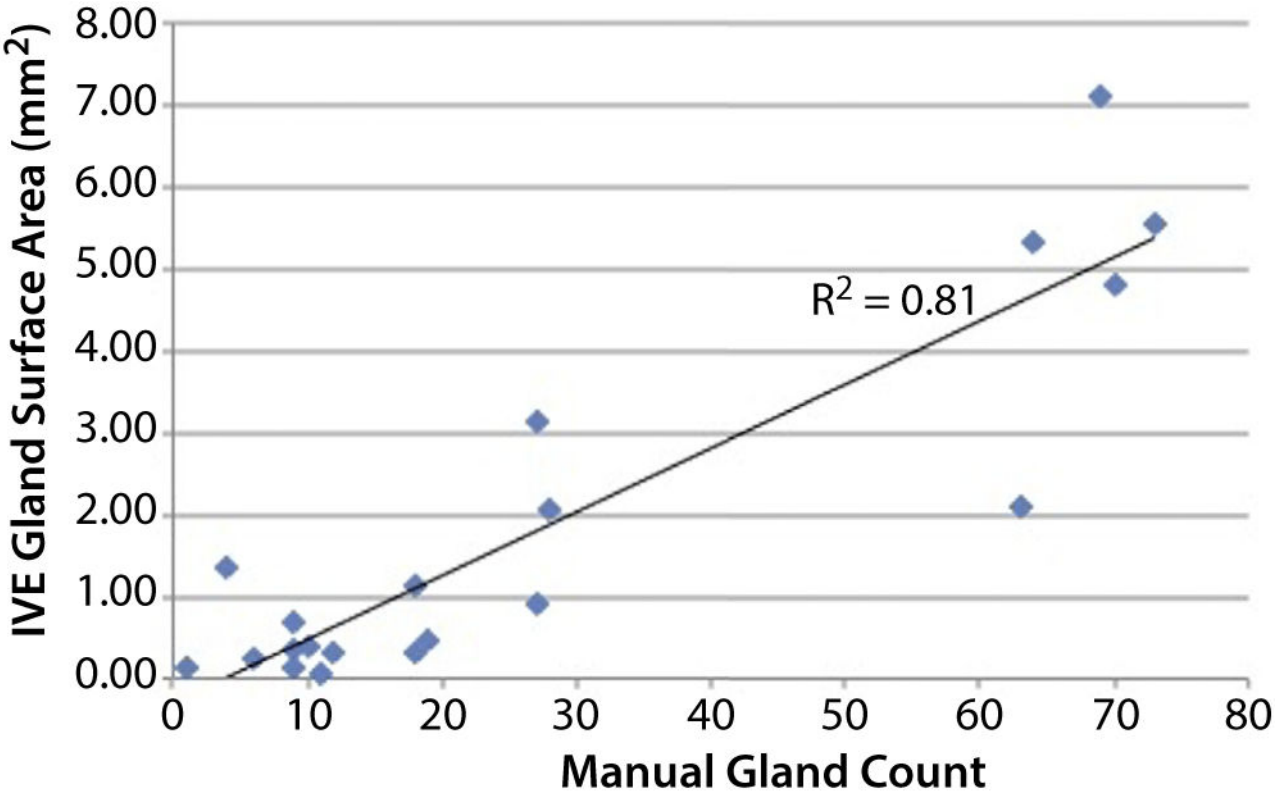

Figure 5.

Association between manual gland quantification and volumetric laser endomicroscopy (VLE) epithelial gland surface area measured using image visualization enhancement software on EMR specimens imaged with the second-generation Nvision VLE Imaging System.

TABLE 1.

Volumetric laser endomicroscopy and histopathology features of EMR specimens with en-bloc (R0) and incomplete (R1) resection

	R0 EMR specimens (n = 14)*	R1 EMR specimens (n = 23)*	*P* value
Volumetric laser endomicroscopy metrics			
Manual gland count, mean (SD)	13.0 (6.7)	28.8 (23.9)	02
Manual gland count per VLE frame, mean (SD)	0.06 (0.03)	0.14 (0.11)	.02
Glands present at margin, n (%)†	5 (20)	20 (80)	<.001
Gland surface area (mm^2), mean (SD)‡	0.98 (1.1)	2.3 (2.4)	.26
Histopathology			.56
Low-grade dysplasia, n (%)	5 (36)	7 (30)	
High-grade dysplasia, n (%)	7 (50)	12 (53)	
Intramucosal adenocarcinoma, n (%)	2 (14)	4 (17)	

SD, Standard deviation; *VLE*, volumetric laser endomicroscopy.

*R0 (en-bloc) resection was defined by the absence of dysplasia and/or cancer at the deep and lateral margins. R1 (incomplete) resection was defined by the presence of dysplasia and/or cancer at the deep and lateral margins.

†A total of 25 EMR specimens showed epithelial glands present at the resection margins.

‡Automated surface area analysis was performed on 20 of 22 EMRs imaged using the second-generation Nvision VLE Imaging System.