

Endoscopic Diagnosis of Invasion Depth for Early Colorectal Carcinomas

A Prospective Comparative Study of Narrow-Band Imaging, Acetic Acid, and Crystal Violet

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Abstract: Several studies have validated the effectiveness of narrow-band imaging (NBI) in estimating invasion depth of early colorectal cancers. However, comparative diagnostic accuracy between NBI and chromoendoscopy remains unclear. Other than crystal violet, use of acetic acid as a new staining method to diagnose deep submucosal invasive (SM-d) carcinomas has not been extensively evaluated. We aimed to assess the diagnostic accuracy and interobserver agreement of NBI, acetic acid enhancement, and crystal violet staining in predicting invasion depth of early colorectal cancers.

A total of 112 early colorectal cancers were prospectively observed by NBI, acetic acid, and crystal violet staining in sequence by 1 expert colonoscopist. All endoscopic images of each technique were stored and reassessed. Finally, 294 images of 98 lesions were selected for evaluation by 3 less experienced endoscopists.

The accuracy of NBI, acetic acid, and crystal violet for real-time diagnosis was 85.7%, 86.6%, and 92.9%, respectively. For image evaluation by novices, NBI achieved the highest accuracy of 80.6%, compared with that of 72.4% by acetic acid, and 75.8% by crystal violet. The kappa values of NBI, acetic acid, and crystal violet among the 3 trainees were 0.74 (95% CI 0.65–0.83), 0.68 (95% CI 0.59–0.77), and 0.70 (95% CI 0.61–0.79), respectively.

For diagnosis of SM-d carcinoma, NBI was slightly inferior to crystal violet staining, when performed by the expert endoscopist. However, NBI yielded higher accuracy than crystal violet staining, in terms of less experienced endoscopists. Acetic acid enhancement

with pit pattern analysis was capable of predicting SM-d carcinoma, comparable to the traditional crystal violet staining.

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Abbreviations: CI = confidence interval, CP = capillary pattern, EMR = endoscopic mucosal resection, ESD = endoscopic submucosal dissection, NBI = narrow-band imaging, NPV = negative predictive value, PP = pit pattern, PPV = positive predictive value, SM-d = deep submucosal invasive, SM-s = superficial submucosal.

INTRODUCTION

With advancements in endoscopic techniques, early colorectal cancers with no risk of lymph node metastasis can be removed endoscopically, such as endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD). It has been demonstrated that lymph node metastasis rarely occurs in intramucosal cancers and superficial submucosal (SM-s) cancers. In contrast, deep submucosal invasive (SM-d) carcinomas have a lymph node metastasis rate of 6% to 12%, and should be treated with surgery.^{1,2} Therefore, assessment of invasion depth of early colorectal cancers is important to make proper therapeutic decision.

Magnifying chromoendoscopy has been generally regarded as the most reliable method for predicting submucosal invasion depth, based on the pit pattern (PP) classification proposed by Kudo.^{3–5} Besides the commonly used dye of indigo carmine and crystal violet, acetic acid can also enhance the visualization of the PP by aceto-white reaction. However, up to now there are few studies investigating the role of this dye and there is controversy on its exact effect.^{6,7} In our pilot study, we found that acetic acid would not cause stain retention, which differs from indigo carmine, especially in those depressed areas.

Narrow-band imaging (NBI) is a relatively novel optical technique which shortens the wavelength of the illumination light by using optical filters. It highlights superficial microcapillaries without the necessity of dye spraying.^{8,9} It is both convenient and time saving as it could be switched between white light and NBI view instantaneously just by touching a button. In published studies, NBI technique yielded equivalent accuracy to chromoendoscopy in differentiating neoplastic from nonneoplastic lesions.^{10–12} However, its value in predicting submucosal invasion depth remains questionable.^{13–15} Therefore, we performed this prospective study to evaluate the diagnostic accuracy of NBI and acetic acid, compared with crystal violet.

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METHODS

Patients and Study Design

The study was conducted prospectively in the department of gastroenterology at Shanghai Renji Hospital from January 2011 to September 2014. Consecutive patients with lesions considered as early colorectal cancers detected by conventional colonoscopy were enrolled. Patients with chronic inflammatory bowel disease, insufficient bowel preparation, familial adenomatous polyposis were excluded from the study. Grossly obvious advanced cancers were also excluded. Lesions diagnosed histopathologically as adenomas were crossed out. The protocol was approved by the medical ethics committee of our hospital, and written informed consents for diagnosis and treatment were obtained from all patients prior to the procedures.

The study was conducted in the following 2 phases.

Phase 1: Real-Time Diagnosis by Endoscopic Expert

Patients were prepared with 2L of polyethylene glycol solution in the morning before the colonoscopy. All colonoscopies were performed by 1 expert with experience of more than 1000 cases in NBI and chromoendoscopy, using a magnifying colonoscope (CF-H260AZI; Olympus Medical systems, Tokyo, Japan) and an electric endoscopic system (EVIS 260 Spectrum; Olympus Medical systems). Lesions were detected by white light endoscopy and then evaluated by NBI, acetic acid, and crystal violet staining in sequence. Tumors having more than one of the following characteristics were suspected of early

cancers and included in this study: depression, hardness, erosion, surface nodularity, convergence of mucosal folds, and mucosal friability.^{16,17} Following conventional view, lesions were observed by magnifying NBI for capillary pattern (CP) analysis. Subsequently, 1.5% acetic acid solution was injected from the forceps channel to stain the mucosa. After PP enhancement by acetic acid had disappeared, 0.05% crystal violet solution was sprayed. PP was evaluated according to Kudo's classification. For each lesion, endoscopic assessments of CPP and PP were recorded. Representative endoscopic images of each method of the same portion were taken and stored (Figures 1 and 2).

For lesions identified as CP IIIA or PP V_I-low, snare polypectomy, EMR, or ESD was performed. For lesions identified as CP IIIB or PP V_I-high/V_N, surgery was proposed. If the diagnoses with NBI and chromoendoscopy were inconsistent, the worse one was adopted for selecting therapeutic strategy. All resected lesions were examined by an independent gastrointestinal pathologist who was blinded to the endoscopic analysis. Histological diagnosis was determined according to the Revised Vienna Classification of Gastrointestinal Neoplasia.¹⁸ Massively invasive submucosal cancers were defined as those with invasion depth of 1000 μm or more.

Phase 2: Image Evaluation by Novices

All images of lesions in phase 1 were collected and reviewed by 2 endoscopic experts. For each lesion, the best quality images of each modality (NBI, acetic acid, and crystal violet) were selected by expert colonoscopists. Images were divided into 3 sets

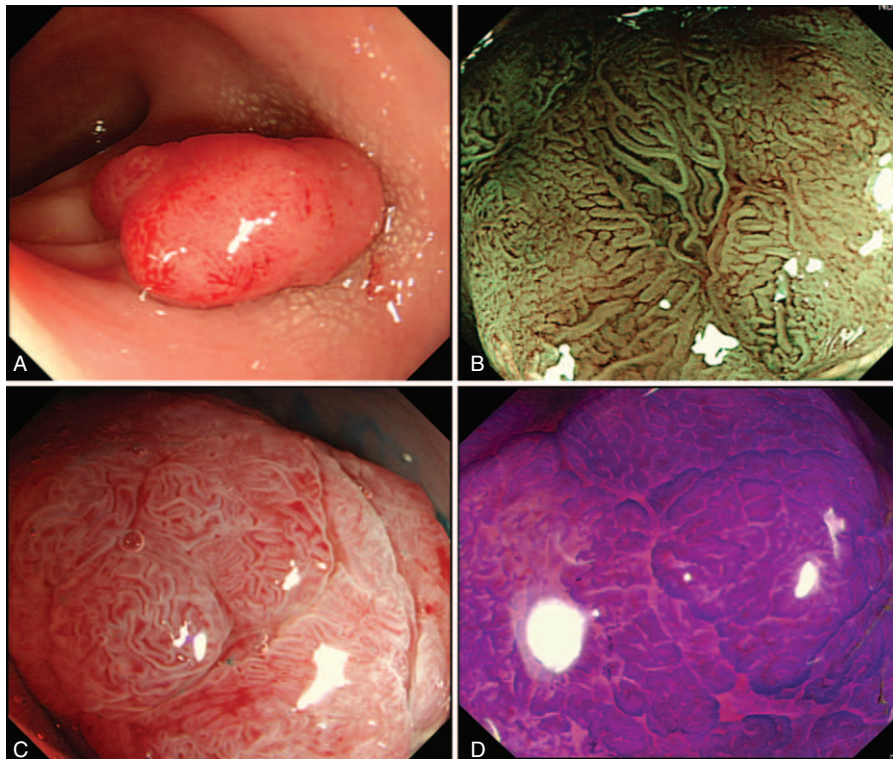


FIGURE 1. A case of colorectal high-grade intraepithelial neoplasia viewed by each modality with magnifying endoscopy. (A) Conventional colonoscopy view. (B) Narrow-band imaging observation revealed high microvessel density with lack of uniformity indicated of capillary pattern type IIIA. (C) Acetic acid enhancement showed dendritic or gyrus-like pit indicative of pit pattern type IV. (D) Crystal violet staining also revealed noninvasive pit pattern of type IV.

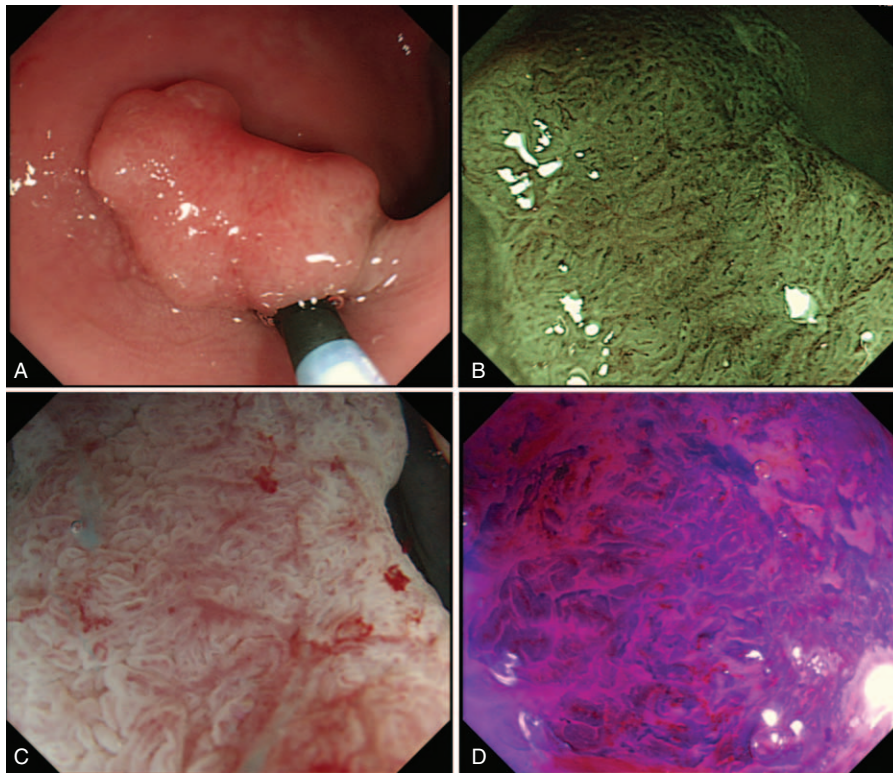


FIGURE 2. Endoscopic images of a colorectal lesion histologically confirmed as sm2 cancer. (A) Conventional colonoscopy view. (B) Narrow-band imaging view indicated capillary pattern type IIIb, as nearly avascular or loose microvascular areas were observed. (C) Acetic acid enhancement showed an irregular glandular structure indicative of pit pattern type V_I . (D) Crystal violet staining showed highly irregular pits and an amorphous structure.

according to diagnostic modality. For each set, images were numbered and randomly arranged. Conventional images and other information of the lesions were not provided.

Three endoscopists with little experience of magnifying NBI or chromoendoscopy (both less than 50 cases) participated in this phase. Before the image test, 3 participants underwent a 1-hour training program that included explanation of the classification criteria and an atlas of images of each type lesions. They reviewed all images independently within 2 weeks of the training. CPs and PPs were assessed for NBI and chromoendoscopy images, respectively. All reviewers were blinded to the detailed information of each lesion, including previous endoscopic evaluation and histological diagnosis.

Classification Criteria of Capillary Patterns and Pit Patterns

According to Sano classification, CP type III was an indicator of carcinomas and was subclassified into type IIIA and IIIb, respectively, indicative of M/SM-s and SM-d. Type III was defined as demonstrating irregular mesh-like microvascular architecture, with characteristics of irregular size, complex branching, disruption, or irregular winding. Type IIIA showed high microvessel density with lack of uniformity, blind ending, branching, and curtailed irregularly. In type IIIb lesions, nearly avascular or loose microvascular areas were observed, with a clear distinction between normal and cancerous mucosa.^{19,20}

In the Kudo classification, PP type V was classified as type V_I and type V_N . PP type V_I showed an irregular glandular structure, and PP type V_N showed loss or decrease of pits with an amorphous structure.²¹ PP type V_I was subclassified as V_I -low and V_I -high. PP type V_I -high was defined as irregular pits with narrowed lumen, rough margin, unclear boundary, abnormal branching, or dense distribution.²² PP type V_I -low was an indicator for M/SM-s; PP type V_I -high and V_N were indicators for SM-d.

Sample Size Calculations and Statistical Analysis

Based on review of literature, the accuracy of magnifying NBI and chromoendoscopy in estimating invasion depth were assumed to be 85% and 95%, respectively.^{5,14} To have 80% power to detect this 10% difference using 1-sided tests, with a type I error rate of 0.05, it was calculated that a total of 105 lesions would be needed.

Diagnostic accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each technique were calculated in comparison with histological diagnosis. Differences of diagnostic performance between 2 modalities were evaluated using the McNemar test. P value < 0.05 was considered statistically significant. Kappa statistics were calculated to assess interobserver agreement for each modality. Kappa values < 0.4 , $0.41-0.6$, $0.61-0.8$, and > 0.8 were regarded as representing poor, fair, good, and excellent agreement, respectively. SPSS 19.0 statistical analysis software was used for statistical analysis.

RESULTS

Clinicopathologic Features of Colorectal Carcinomas

Of the included 2238 patients with 2156 colorectal lesions, 132 lesions were initially suspected as early colorectal cancers by conventional endoscopy. After histological evaluation, 12 lesions were confirmed as adenomas and 8 lesions were advanced cancers. Thus, a total of 112 early colorectal cancers in 109 patients were analyzed (Figure 3). The mean age of the patients was 59.5 ± 12.3 years. The mean size of the lesions was 23.2 mm (ranged 8–60 mm). Morphologically, there were 37 polypoid, 54 flat elevated, and 21 depressed lesions. Thirty-nine lesions were located in the right-sided colon, 45 lesions were located in the left-sided colon, and 28 were rectal lesions. Upon histological evaluation, there were 31 adenomas with high-grade dysplasia, 34 intramucosal cancers, 13 SM-s cancers, and 34 SM-d cancers.

Diagnostic Accuracy of In Vivo Prediction of Invasion Depth

The sensitivity, specificity, NPV, PPV, and overall accuracy of each modality in predicting deep submucosal invasion were presented in Table 1. The overall accuracy of crystal violet (92.9%) was higher than those of NBI (85.7%) and acetic acid (86.6%), but no statistically significant differences were found. The specificity of NBI was significantly inferior to that of crystal violet (84.6% vs 93.6%, *P* = 0.039). All 3 diagnostic techniques yielded excellent NPV exceeding 90%. In contrast, the PPV of each modality was relatively lower, which was 71.4% by NBI, 74.4% by acetic acid, and 86.5% by crystal violet.

Diagnostic Performance of Novices in Image Evaluation

All endoscopic images of the 112 lesions were reviewed and reassessed by 2 experts. Images of 11 lesions were considered to be of poor quality, due to overlying mucus or out of focus. The 2 experts discussed with each other and reached an agreement on the CP and PP classification for all lesions except 3 lesions. These 14 lesions were excluded from the image

TABLE 1. Diagnostic Performance of Narrow-Band Imaging, Acetic Acid, and Crystal Violet Staining by Expert

	NBI	Acetic Acid	Crystal Violet
Accuracy	85.7% (96/112)	86.6% (97/112)	92.9% (104/112)
Sensitivity	88.2% (30/34)	85.3% (29/34)	94.1% (32/34)
Specificity	84.6% (66/78)*	87.2% (68/78)	93.6% (73/78)*
PPV	71.4% (30/42)	74.4% (29/39)	86.5% (32/37)
NPV	94.3% (66/70)	93.2% (68/73)	97.3% (73/75)

NBI = narrow-band imaging, NPV = negative predictive value, PPV = positive predictive value.

**P* = 0.039, NBI versus crystal violet, calculated by the McNemar test.

evaluation phase. Thus, a total of 294 images of 98 lesions were selected for the image test. Of these lesions, 68 were HGD/M/SM-s, and 30 were SM-d.

Diagnostic accuracy, sensitivity, specificity, PPV, and NPV of each endoscopist were shown in Table 2. Diagnostic accuracy of invasion depth using NBI ranged from 83.7% to 77.6%, while those using acetic acid and crystal violet ranged from 76.5% to 68.4% and 79.6% to 72.4%, respectively. Combined performances of the 3 endoscopists for each endoscopic modality were summarized in Table 3. NBI yielded the highest accuracy, sensitivity, specificity, PPV, and NPV among the 3 modalities. The accuracy and specificity of NBI were significantly higher than those of acetic acid (*P* = 0.02 and 0.023, respectively). PP analysis using acetic acid and crystal violet staining had similar performance.

The kappa values for interobserver agreement for NBI, acetic acid, and crystal violet were 0.74 (95% CI 0.65–0.83), 0.68 (95% CI 0.59–0.77), and 0.70 (95% CI 0.61–0.79), all showing good agreement without significant difference between endoscopic modalities.

DISCUSSION

Several techniques have been utilized to improve the diagnostic accuracy of early colorectal cancers to avoid unnecessary surgery. In this study, we evaluated the accuracy

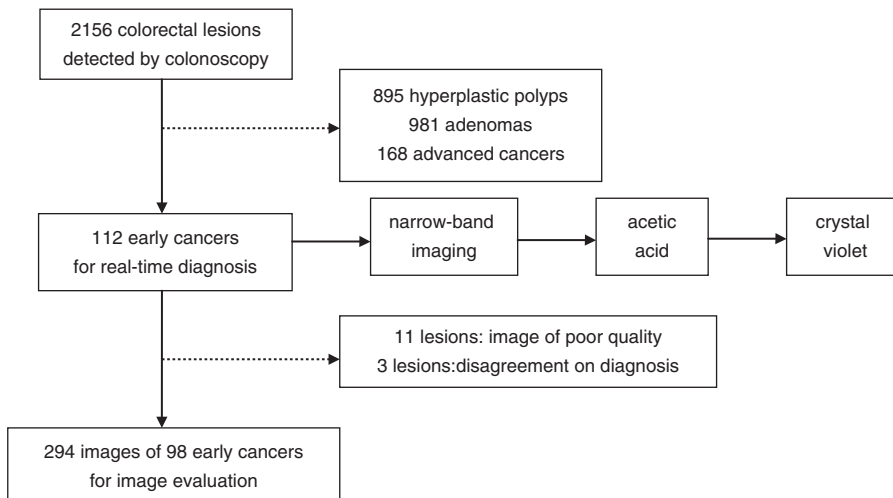


FIGURE 3. Flow chart of patient selection in this study.

TABLE 2. Performance Characteristics of Each Inexperienced Endoscopists (A, B, and C) for Invasion Depth in Endoscopic Images

	NBI			Acetic Acid			Crystal Violet		
	A	B	C	A	B	C	A	B	C
Accuracy (%)	83.7	77.6	80.6	76.5	72.4	68.4	79.6	75.5	72.4
Sensitivity (%)	86.7	80	83.3	83.3	80	73.3	83.3	80	77
Specificity (%)	82.4	75.6	79.4	73.5	69.1	66.2	77.9	73.5	70.6
PPV (%)	68.4	60	64.1	58.1	53	48.9	62.5	57.1	54
NPV (%)	93.3	89.7	91.5	90.9	88.7	84.9	91.4	89.3	87.3

NBI = narrow-band imaging, NPV = negative predictive value, PPV = positive predictive value.

and reliability of NBI, acetic acid, and crystal violet, not only for real-time diagnosis by expert, but also for image evaluation by inexperienced endoscopists.

We used crystal violet instead of indigo carmine, as it can provide more detailed PP structure for PP type V lesions. Besides, the evaluation of PP would sometimes be difficult due to the accumulation of indigo carmine solution in the depressed area. Matsuda et al⁵ reported that the sensitivity and specificity of PP analysis for diagnosis of invasion depth were 85.6% and 99.4%, respectively. Nevertheless, crystal violet solution may have potential toxicity as it can be absorbed into epithelial cells and bind the nuclei.²³ In the present study, crystal violet staining performed by experienced colonoscopy achieved high accuracy of 92.9%. However, diagnostic accuracy of inexperienced endoscopists was relatively unsatisfactory, which implied that a short training session was not enough for novices to reach expert level. Sometimes it was difficult for novices to subclassify lesions with type VI PP, as this type included various lesions from dysplasia to SM-d.

Acetic acid can remove the adherent mucus and improve PP visualization through the process known as ‘‘aceto-white reaction.’’ The primary mechanism is that acetic acid breaks the disulfide bonds of glycoproteins in the mucus and causes reversible denaturation of intracellular cytoplasmic protein.^{24,25} The enhancement of PP by acetic acid persists only several minutes, so it had no influence on the subsequent crystal violet staining. For diagnosis of deep submucosal invasion in colorectal carcinoma, Kawamura et al showed that acetic acid had a sensitivity of 73.3% and a specificity of 94.4%, similarly to those of indigo carmine.²⁶ A recent study demonstrated that magnifying NBI with acetic acid enhancement was comparable to chromoendoscopy with crystal violet in PP diagnosis of colorectal neoplasms.²⁷ However, the number of early colorectal cancers in the previous 2 studies was rather small. Our

study was the first large-scale study on the application of acetic acid spraying in predicting invasion depth. We found that acetic acid enhancement achieved slightly inferior diagnostic performance with crystal violet, without statistically significant difference. Washing with acetic acid repeatedly would sometimes lead to slight bleeding on the surface of the lesions. During our study period, no adverse events of acetic acid spraying were found.

Despite high accuracy, chromoendoscopy is operator dependent and labor intensive, which had restricted its widespread use especially in Western countries. With the advantage of convenience and simplicity, the innovative NBI technique yielded high accuracy and good interobserver agreement equivalent to chromoendoscopy in differentiating adenomas from hyperplastic polyps.^{28,29} However, it is considered to be less accurate for the prediction of deep submucosal invasion. It has been suggested that conventional PP observation or EUS assessment should be performed for lesions classified as CP type IIIB.^{5,9} A retrospective study using still images indicated that NBI had comparable accuracy but greater interobserver variability than chromoendoscopy in estimating invasion depth of early colorectal cancers.³⁰

In our study, magnifying NBI performed by 1 expert achieved high accuracy of 85.7%, comparable to that of chromoendoscopy. The specificity of NBI was significantly inferior to crystal violet, which revealed potential tendency to overstage. For the 42 lesions identified as CP type IIIB, 12 lesions were M/SM1, which could be resected endoscopically. After crystal violet staining, 6 of the 12 lesions were judged to be PP type V₁. Therefore, chromoendoscopy may be necessary for some lesions identified as CP type IIIB. These 2 techniques might compensate for each other, which could contribute to more precise diagnosis of invasion depth. After a 1-hour training program, inexperienced endoscopists achieved an overall accuracy of 80.6%, sensitivity

TABLE 3. Combined Performance Characteristics of Inexperienced Predictions of Deep Submucosal Invasion in Still Images

	NBI Mean (95% CI)	Acetic Acid Mean (95% CI)	Crystal Violet Mean (95% CI)
Accuracy	80.6%* (72.8%–88.5%)	72.4%* (63.6%–81.3%)	75.8% (67.4%–84.3%)
Sensitivity	83.3% (75.9%–90.7%)	78.9% (70.8%–87.0%)	80.0% (72.1%–87.9%)
Specificity	79.1%† (71.1%–87.2%)	69.6%† (60.5%–78.7%)	74.0% (65.3%–82.7%)
PPV	64.2% (54.7%–73.7%)	53.4% (43.6%–63.3%)	57.7% (47.9%–67.5%)
NPV	91.5% (86.0%–97.0%)	88.2% (81.8%–94.6%)	89.3% (83.2%–95.4%)

CI = confidence interval, NBI = narrow-band imaging, NPV = negative predictive value, PPV = positive predictive value.

* *P* = 0.02 NBI versus AA.

† *P* = 0.023 NBI versus AA.

of 83.3%, and specificity of 79.1%, which were higher than those of crystal violet spraying. These results indicated that NBI is an easier to learn diagnostic tool than chromoendoscopy. However, to achieve accurate estimation of invasion depth, sufficient experience is required for both NBI and chromoendoscopy.

This study did have several limitations. Because submucosal invasive colorectal cancers are uncommon, every lesion was observed by all 3 modalities in sequence, instead of randomly assigned to any 1 modality. Thus, the former procedure probably made some influence on the latter technique. Second, all the real-time endoscopic assessment of carcinomas was performed by 1 highly experienced endoscopist. Additionally, the study was conducted in a single medical center, with limited number of endoscopists.

In conclusion, our results suggested that NBI and acetic acid had similar accuracy and interobserver agreement in comparison with crystal violet for estimating invasion depth of early colorectal neoplasms. Acetic acid enhancement is an effective and simple method to diagnose submucosal cancers. Compared with chromoendoscopy, NBI is more convenient, free of dye spraying, and easy to learn, showing its prospects in clinical practice. Further multicenter prospective studies with the combination of these endoscopic modalities are needed to establish the optimal strategy for predicting invasion depth of early colorectal cancers.

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