

Rectal Optical Markers for *In Vivo* Risk Stratification of Premalignant Colorectal Lesions

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Abstract

Purpose: Colorectal cancer remains the second leading cause of cancer deaths in the United States despite being eminently preventable by colonoscopy via removal of premalignant adenomas. In order to more effectively reduce colorectal cancer mortality, improved screening paradigms are needed. Our group pioneered the use of low-coherence enhanced backscattering (LEBS) spectroscopy to detect the presence of adenomas throughout the colon via optical interrogation of the rectal mucosa. In a previous *ex vivo* biopsy study of 219 patients, LEBS demonstrated excellent diagnostic potential with 89.5% accuracy for advanced adenomas. The objective of the current cross-sectional study is to assess the viability of rectal LEBS *in vivo*.

Experimental Design: Measurements from 619 patients were taken using a minimally invasive 3.4-mm diameter LEBS probe introduced into the rectum via anoscope or direct insertion, requiring approximately 1 minute from probe insertion to with-

drawal. The diagnostic LEBS marker was formed as a logistic regression of the optical reduced scattering coefficient μ_s^* and mass density distribution factor D .

Results: The rectal LEBS marker was significantly altered in patients harboring advanced adenomas and multiple non-advanced adenomas throughout the colon. Blinded and cross-validated test performance characteristics showed 88% sensitivity to advanced adenomas, 71% sensitivity to multiple non-advanced adenomas, and 72% specificity in the validation set.

Conclusions: We demonstrate the viability of *in vivo* LEBS measurement of histologically normal rectal mucosa to predict the presence of clinically relevant adenomas throughout the colon. The current work represents the next step in the development of rectal LEBS as a tool for colorectal cancer risk stratification. *Clin Cancer Res*; 21(19); 4347–55. ©2015 AACR.

Introduction

The approximately 35% decline in colorectal cancer incidence rate since the mid-1980s is a testament to the huge success of colon cancer screening within the United States (1). In more recent years, much of this decline has been attributed to the increased uptake of the now "gold standard" colonoscopy, which has the ability to not only identify, but also to treat premalignant lesions through direct removal (2). Despite this proven capability, a number of sizeable disadvantages, including high cost, limited endoscopist resources, patient discomfort, and the potential for procedural complications make colonoscopy an imperfect method for use in population-wide screening. Given that colorectal cancer remains the second leading cause of cancer death in the

United States (~50,000 in 2014), improved screening paradigms are therefore still welcome (1).

In order to further reduce colorectal cancer mortality, a two-step paradigm shift in screening has often been proposed. Under this approach, patients within the asymptomatic population would first undergo a minimally invasive prescreen to identify individuals at highest risk of developing colorectal cancer (i.e., patients harboring premalignant lesions). These high-risk individuals would then proceed to a follow-up colonoscopy for removal of all precancerous adenomas. A screening program of this kind could simultaneously optimize patient compliance and resource allocation in order to more effectively protect against the development of fatal colorectal cancer. Moreover, evidence of the effectiveness of such an approach is demonstrated in the tremendously successful Pap test—colposcopy screening paradigm for cervical cancer.

To ensure maximum efficacy as well as patient compliance, the prescreening technique would need to be highly accurate at detecting premalignant lesions throughout the colon, pose a minimal amount of patient discomfort/harm, and have the ability to be carried out in the primary care setting or via at-home tests. Unfortunately, alternative imaging-based screening techniques such as flexible sigmoidoscopy and virtual colonoscopy cannot meet all of these stringent requirements (3). Another option that overcomes some of the limitations of imaging-based techniques is to implement less-invasive fecal tests. Given the low prevalence of colorectal cancer in the general screening population (~0.7%; ref. 4) and that colonoscopy already possesses the ability to accurately detect and remove precancerous adenomas throughout

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Translational Relevance

Our multidisciplinary group identifies patients at risk of developing cancer through application of the well-established concept of field carcinogenesis—the idea that genetic/epigenetic changes diffusely spread throughout an organ create a "fertile field" from which frank cancer may develop. The implications of field carcinogenesis on cancer screening are 2-fold: First, it enables the early detection at a time point where preventative measures are most effective. Second, because the field is spread throughout an organ, a patient's risk level can be assessed through easily accessible surrogate tissue sites. Low-coherence enhanced backscattering (LEBS) is a 3.4-mm diameter fiber-optic probe technology that detects the structural consequences of colorectal field carcinogenesis through *in vivo* interrogation of the rectal mucosa. From a translational perspective, we envision the future use of LEBS applied in the primary care setting during an annual physical exam to identify those patients who would most benefit from a colonoscopy.

the colon, the primary mandate of any colorectal cancer screening paradigm must be to identify patients with precancerous lesions (as recommended by the American Cancer Society, the U.S. Multi Society Task Force on Colorectal Cancer, and the American College of Radiology; ref. 5). Yet all currently available fecal tests exhibit a lack of sensitivity to such treatable lesions. For example, while fecal occult blood test (FOBT), fecal immunochemical test (FIT), and stool DNA test (sDNA) have demonstrated excellent sensitivity to cancerous lesions, they provide low test sensitivity to precancerous advanced adenomas [10.8%, 29.5%, and 42.4% sensitivity for FOBT (6, 7), FIT (7), and sDNA (4) respectively].

In an effort to improve upon existing screening tests, our multidisciplinary group focuses on identifying high-risk patients through application of the well-established concept of field carcinogenesis (8–14). Field carcinogenesis is the very initial stage of cancer progression in which a number of genetic/epigenetic mutations [e.g., DNA methylation (9), histone modifications (15), and altered cytoskeletal mediators of chromosomal stability (16)] spread diffusely throughout an organ due to common environmental insult and genetic composition (17). The resulting structural correlates of such mutations are subtle intracellular and extracellular alterations occurring at ultra structural length-scales (i.e., <~300 nm; ref. 18). Taken together, the combined contribution from these mutational and structural alterations composes a fertile "field of injury" from which focal arise via stochastic mutational events such as mutation of key oncogenes (e.g., *KRas*) and tumor suppressor genes (e.g., *p53*, *adenomatous polyposis coli APC*; ref. 17). In clinical practice, field carcinogenesis provides the basis upon which flexible sigmoidoscopy is able to predict synchronous proximal neoplasia through inspection of the distal colon (17, 19). Moreover, our group has previously applied field carcinogenesis to optically detect colorectal adenomas throughout the colon via rectal measurements (17, 20–26).

Because the ultrastructural alterations occurring in field carcinogenesis are by definition smaller than the resolution limit of optical microscopy, the mucosa appears histologically normal under conventional endoscopy/histology. Our group therefore

invented a novel optical spectroscopy technique known as low-coherence enhanced backscattering (LEBS) capable of selectively targeting the subtle ultrastructural changes occurring in colon field carcinogenesis (22, 27–29). In a previous *ex vivo* LEBS study of rectal biopsies, our group confirmed the presence of ultrastructural alterations occurring at depths within the first 400 to 600 μm of colonic mucosa (22). Moreover, we demonstrated that these optical changes contained excellent diagnostic potential with an overall accuracy of 89.5% for advanced adenomas (21). In order to translate these findings into the clinic for *in vivo* application, we developed a minimally invasive 3.4-mm diameter fiber-optic LEBS probe optimized for detecting the previously observed changes (30). We therefore hypothesize that the changes previously measured *ex vivo* should also be detectable *in vivo*. In this paper, we determine the feasibility of *in vivo* LEBS measurement of histologically normal rectal mucosa to predict the presence of precancerous adenomas throughout the colon on a cross-sectional study of 619 subjects.

Materials and Methods

Participants and acquisition of clinical data

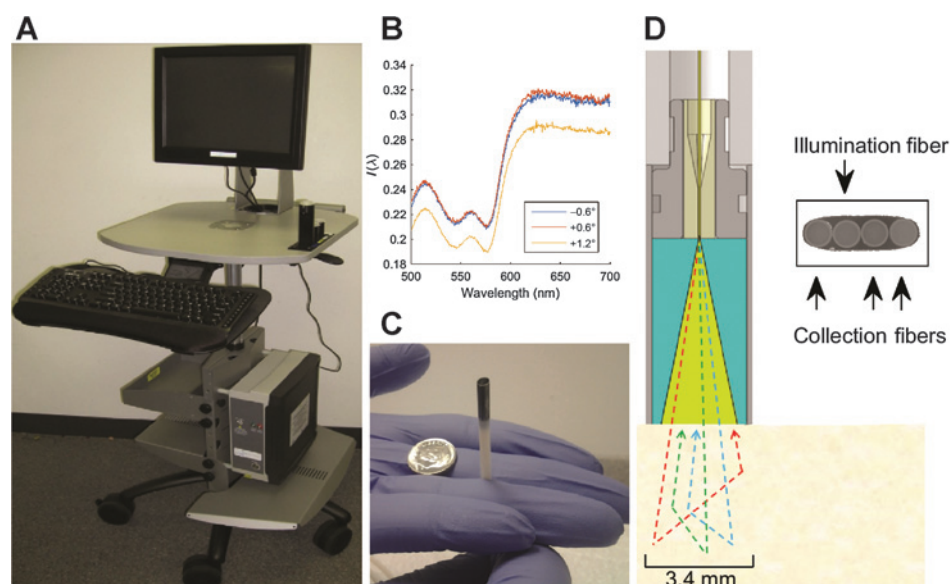
This cross-sectional study was approved by the Institutional Review Boards at NorthShore University HealthSystem (Evanston, IL), Indiana University School of Medicine (Indianapolis, IN), and the University of Chicago Medical Center (Chicago, IL). Patients were eligible for recruitment into the study if they were already scheduled for population-based colonoscopy screening or surveillance as recommended by their general practitioner or gastroenterologist. Seven hundred and twenty-eight asymptomatic patients that were free of cancer were recruited into the study after providing written informed consent. Six hundred and nineteen of these patients had completed data work-ups from an endoscopic findings report, a pathology report, a demographic information survey, and met the criteria for robust LEBS measurements discussed below. Six hundred and two of these patients were recruited for the main study, while the remaining 17 were recruited for a separate pilot study to assess the feasibility of performing LEBS in patients without colon preparation (further details found below).

All measurements were acquired through the point-of-care instrument shown in Fig. 1A (assembled by Tricor Systems). Following the withdrawal phase of colonoscopy, the 3.4-mm diameter LEBS probe was introduced into the rectal vault via direct insertion or anoscope. The endoscopist then took 10 readings from random locations within the rectum, applying gentle contact with the tissue surface. Each reading took less than 0.5 seconds and recorded the backscattered light intensity collected at three angles as shown in Fig. 1B. The entire procedure from probe insertion to extraction typically took less than 1 minute. Measurements were acquired by trained endoscopists and the final data analysis was performed in post-processing by the investigators using automated data analysis algorithms. At the time of data acquisition, endoscopists were blinded to the pathology findings of polyps retrieved during the preceding colonoscopy. Four distinct endoscopists were used in this study. Histopathology reports were prepared by each study site with no centralized pathologic review being performed.

In order to ensure the maximum level of patient compliance, LEBS will need to be performed in patients not subjected to colon preparation. To that end, a pilot study of 17 patients was asked to

Figure 1.

Clinical LEBS probe and point-of-care instrumentation used for data collection. A, mobile point-of-care system housing the data acquisition instrumentation, calibration mechanism, and custom operating software. B, backscattering light spectrum measured by the LEBS probe at 3 collection angles: -0.6° , $+0.6^\circ$, and $+1.12^\circ$ relative to the incident direction. C, demonstration of the scale of the LEBS probe relative to the hand of a clinician. Rectal LEBS measurements are taken via direct insertion into the colon. D, schematic showing the 3.4-mm diameter fiber-optic LEBS probe. The inset shows an image of the linear optical fiber array, with sample illumination via one of two inner fibers and collection of backscattered light via the remaining fibers.



undergo LEBS measurement at a date before colonoscopy in which they were not required to follow any preparation regimen (e.g., no bowel purge, dietary restrictions, etc.). The follow-up screening colonoscopy was then performed according to standard protocols within one month of the initial LEBS measurement.

For diagnostic purposes, patients were classified into four categories: no dysplasia (control), 1 or 2 non-advanced adenomas with size ≤ 9 mm, multiple (3 or more) non-advanced adenomas, and advanced adenomas (size ≥ 10 mm, $\geq 25\%$ villous features, or high-grade dysplasia). Because of their clinical relevance to colorectal cancer progression, the primary endpoint of our study was the identification of advanced adenomas. Adenoma size was specified as the largest polyp dimension reported on either the colonoscopy findings or pathology report. Polyp histomorphology (e.g., hyperplastic, adenomatous, and/or serrated) was determined based on pathology findings. We note that serrated histomorphology had no significant effect on our LEBS measurements—thus adenomas were grouped based solely on the criteria above. Diagnostic categorization was performed at Northwestern University with disagreements resolved by a consensus of at least two senior investigators.

LEBS fiber optic probe instrumentation and biomarkers

The principles behind the fiber-optic LEBS probe shown in Fig. 1C have previously been described in other publications (30, 31). In short, the LEBS probe (assembled by OFS; schematic in Fig. 1D) consists of four optical fibers terminated in a rigid housing that aligns the fibers into a linear array (see inset of Fig. 1D). One of the central fibers delivers white light illumination from a xenon lamp onto the tissue surface (<1 mW delivered to tissue), whereas the remaining fibers collect backscattered light at three angles (-0.6° , $+0.6^\circ$, and $+1.12^\circ$ relative to the incident direction). The backscattered light is collected by a spectrometer-based system designed to collect visible light between 500 nm and 700 nm. In order to control the spatial coherence (L_{sc}) of the illumination and thereby limit the tissue depths that are interrogated, the illumination fiber core diameter is set to $50 \mu\text{m}$ and a 9-mm glass rod separates the fiber array from the tissue surface. This configuration results in $L_{sc} = 27 \mu\text{m}$ at 700 nm illumination

corresponding to superficial penetration depths between approximately 100 to $150 \mu\text{m}$ (30, 32).

LEBS analysis of tissue ultra-structure

A full description of the optical quantification of tissue ultra-structure can be found in other publications (22, 29, 33). Here, we briefly review the basic aspects that are pertinent for the current study.

Colon mucosa is composed of a wide variety of arbitrarily shaped structures ranging in size from tens of nanometers (e.g., cell membranes, histones, and cytoskeleton) to hundreds of nanometers and microns (e.g., mitochondria, nuclei, and collagen fibers) to hundreds of microns (e.g., colon crypts) in size. In order to quantify the spatial organization of such heterogeneous structures, we use two bulk property parameters: the variance of mass density σ_ρ^2 and the mass density distribution factor D . Through application of scattering theory, LEBS can directly measure D and indirectly measure σ_ρ^2 using the reduced scattering coefficient μ'_s as a proxy (i.e., $\mu'_s \propto \sigma_\rho^2$; refs. 34, 35).

Data processing and statistical analysis

All data processing and statistical analysis were performed using MATLAB version R2013A. Furthermore, the analysis included in the current work was performed at a time point after all training and validation data had been collected in the clinic.

The optical properties D and μ_s^* are calculated using relationships derived in other publications (34, 35). D is calculated by fitting the power-law decay of diffusely scattered light intensity (measured as the spectrum of light collected at $+1.12^\circ$, see Fig. 1B) as a function of wavelength: $I(\lambda) \propto \lambda^{D-4}$. To calculate μ_s^* , we make the assumption that the anisotropy factor $g = 0.9$ in colonic mucosa (36). We then use empirical relations derived using Monte Carlo simulation to calculate $\mu_s^* = -7133 \text{ cm}^{-1} \cdot E' + 3762.5 \text{ cm}^{-1} \cdot E' \cdot D - 4.53 \text{ cm}^{-1}$ (35), where E' is the average of the intensities measured at -0.6° and $+0.6^\circ$ minus the intensity at $+1.12^\circ$ (30, 35). Before applying these equations, the value of E' was first multiplied

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by a constant calibration factor of 0.31 to achieve agreement between experiment and Monte Carlo simulation.

Various data exclusion criteria were implemented to guarantee data robustness. First, we removed all measurements that resulted in $E' < 0$. Physically speaking E' must be greater than 0, thus negative E' are indicative of aberrant measurements (e.g., poor probe contact pressure, regions of poor colon prep, or damaged tissue). We then excluded patients with values of D that were determined to be unphysical for rectal mucosa (e.g., $D > 6$). Next, we removed individual measurements of E' and D whose values were outside of the range $[Q_1 - 1.5(Q_3 - Q_1), Q_3 + 1.5(Q_3 - Q_1)]$, where Q_1 and Q_3 are the 25th and 75th percentiles, respectively. After applying these criteria and provided that there was a minimum of three usable measurements, the remaining measurements were averaged together to arrive at a final average patient measurement. We subsequently applied this same criterion to remove entire patients with abnormally high or low marker readings relative to their peers. In total, 93 subjects were removed due to non-robust measurement.

In order to generate a single diagnostic biomarker (termed the LEBS marker), we combined D and μ_s^* using multivariate logistic regression. To ensure the robustness of the LEBS marker prediction rule, we chronologically separated the first 80% of our dataset into a training set ($N = 476$) and the final 20% into a blinded validation set ($N = 126$). The prediction rule was then optimized between healthy controls and patients harboring advanced adenomas:

$$\text{LEBS marker} = a_0 + a_1D + a_2\mu_s^* \quad (1)$$

where a_n are coefficients assigned using the function "mnrfit" applied to patients in the training set. The optimal LEBS marker threshold (threshold = -2.35) was then determined by maximizing the sensitivity and specificity of the training set (i.e., finding the point closest to sensitivity = specificity = 1.0). Applying the logistic regression coefficients and LEBS marker threshold value to the validation set, we calculated the sensitivity, specificity, and the overall accuracy of the test by generating the receiver operating characteristic (ROC) curve using the function "perfcurve." The 95% confidence intervals on the sensitivity and specificity were calculated using the binomial proportion confidence interval, while for the overall accuracy, they were calculated according to equations by Hanley and McNeil (37). Investigation into the contribution from confounding factors (e.g., age, race, smoking/alcohol status, and personal and family history of adenomas/cancer) was evaluated by performing an analysis of covariance (ANCOVA) test using the function "anovan."

Results

Patient characteristics

Figure 2 diagrams the various patient groupings used in the current study. LEBS readings from 619 patients (age 58.4 ± 11.0 years; 60.0% female; 83.8% Caucasian) were included in our analysis. These 619 patients were separated into the main study of 602 "prepped" patients with standard colon preparation and a pilot study of 17 "unprepped" patients without colon preparation. In order to ensure the robustness of our unprepped diagnostic marker, the first 476 patients (age 58.1 ± 11.5 years; 61.7% female; 82.6% Caucasian) were grouped

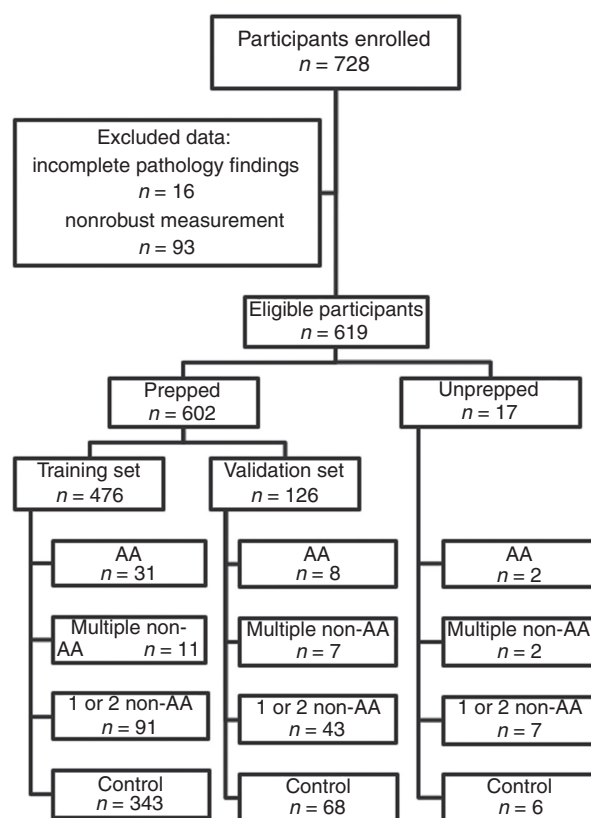


Figure 2.

Diagram and categorization of all enrolled patients. AA, patients with advanced adenomas; non-AA, patients with non-advanced adenomas.

into a prediction rule training set and the final 126 patients (age 58.7 ± 9.22 years; 56.3% female; 85.7% Caucasian) were grouped into an independent validation set. There were no significant demographic differences between the training and validation set.

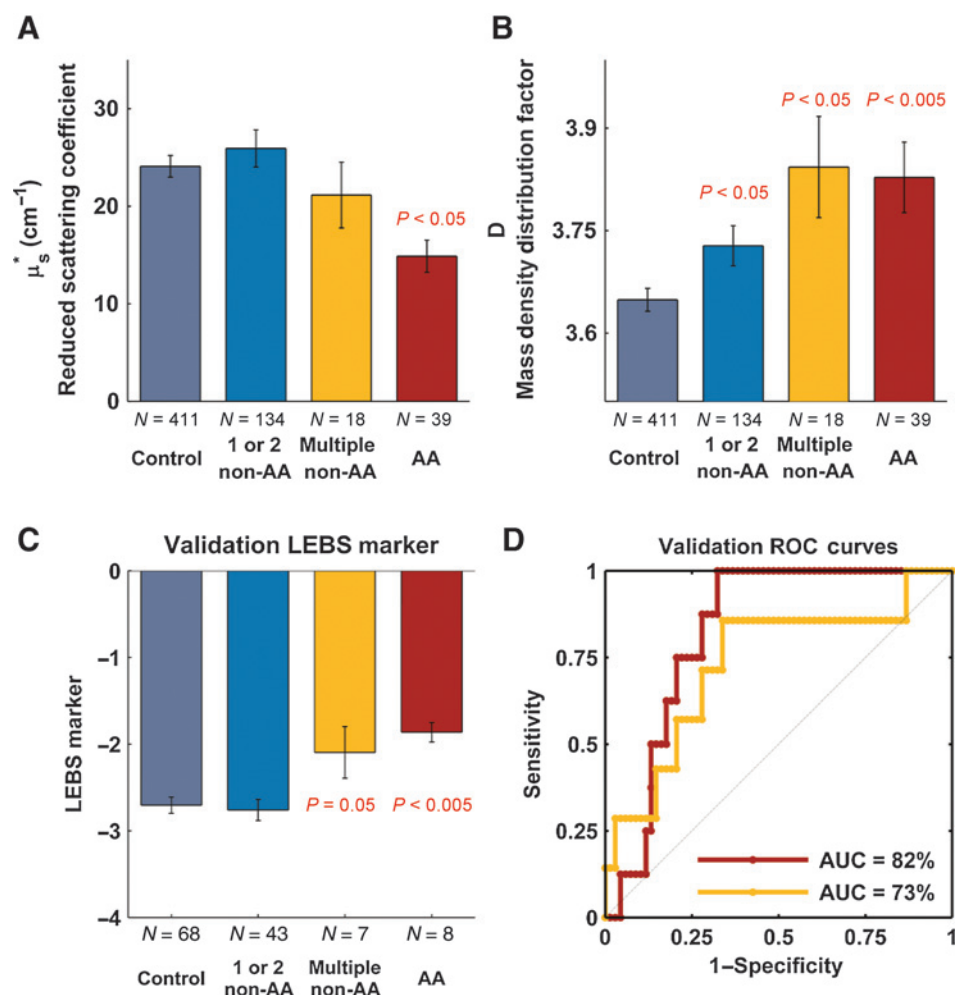
The prediction rule was optimized using the training dataset with 343 healthy control patients versus 31 patients with advanced adenomas. In the training set, the only significant demographic difference was an increase ($P = 0.004$) in the age of patients with advanced adenomas (63.4 ± 9.8 years) relative to control (57.3 ± 11.4 years). We subsequently validated the prediction rule using patients in the validation set with 68 healthy controls versus 8 patients with advanced adenomas and 7 patients with multiple non-advanced adenomas. There were no significant demographic differences between these three validation set patient groupings.

Optical alterations occurring in colorectal cancer field carcinogenesis

Combining the training and validation set, Fig. 3A and B shows the alterations in the optical properties μ_s^* and D , respectively. For μ_s^* , we observed a significant 38.3% decrease in value ($P = 0.012$) between controls and patients with advanced adenomas. There was no significant difference detected in μ_s^* for patients with any number of non-advanced adenomas. For D , there was a progressive increase in value from control to 1 or 2 non-advanced adenoma ($P = 0.019$) to multiple non-advanced adenoma

Figure 3.

Optical alterations of the rectal mucosa in patients exhibiting colorectal cancer field carcinogenesis enables accurate detection of clinically relevant adenomas. A, a significant decrease in the reduced scattering coefficient μ_s^* for patients harboring advanced adenomas. B, a significant increase in the mass density distribution factor D . C, the diagnostic LEBS marker for patients in the validation set. D, the ROC curves for validation set patients. The AUC is 82% for advanced adenomas and 73% for multiple non-advanced adenomas. The x-axis of A–C are ordered according to increasing aggressiveness from left to right. Control, healthy controls; Non-AA, patients with non-advanced adenomas; AA, patients with advanced adenomas.



($P = 0.017$) and advanced adenoma patients ($P = 0.002$). The directionality of these changes ($\downarrow\mu_s^*$ and $\uparrow D$) is consistent with trends observed in three prior studies of rectal mucosa in field carcinogenesis, corroborating the claim that the current study is detecting the same alterations that were previously observed *ex vivo* (21–23).

Diagnostic power of the LEBS marker

The diagnostic LEBS marker was evaluated according to Eq. 1 as a linear combination of μ_s^* and D . As shown in Fig. 3C, the LEBS marker remains unaltered for patients with 1 or 2 non-advanced adenomas, but shows a progressive increase in value for patients with multiple non-advanced adenomas ($P = 0.052$) and those with advanced adenomas ($P = 0.004$). Figure 3D shows two ROC curves generated using the LEBS marker values for patients harboring advanced adenomas and those with multiple non-advanced adenomas. The overall accuracy of detecting advanced adenomas (summarized as the area under the ROC curve) was favorable at 82%. Applying the optimal LEBS marker threshold to the validation set provided 88% sensitivity to advanced adenomas with 72% specificity. For multiple non-advanced adenomas, the diagnostic potential was moderately lower with 71% sensitivity and 73% overall accuracy. Table 1 summarizes the test

performance characteristics of the LEBS marker for the validation dataset.

Potential confounders

To assess the contribution of common colorectal cancer risk factors to our diagnostic results, we carried out an ANCOVA with the LEBS marker as the dependent variable and the presence of advanced adenoma, endoscopist, age, gender, race, smoking history, alcohol history, and personal/family history of adenoma/cancer as predictors (results summarized in Table 2). After including these factors into our model, the LEBS marker remained a highly significant predictor of advanced adenomas with $P < 0.001$. The contributions from all other confounding factors were insignificant except for gender.

Table 1. Validation set test performance characteristics

Adenoma type	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)
Advanced adenoma	88% (65%–100%)	72% (61%–83%)	82% (70%–94%)
Multiple non-advanced adenomas	71% (37%–100%)	As above	73% (58%–89%)

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Table 2. Confounding factor analysis

Factor	P
Advanced adenoma	<0.001
Endoscopist	0.80
Age	0.95
Gender	<0.001
Race	0.71
Smoking	0.94
Alcohol	0.96
Personal history of adenoma or colon cancer	0.13
Family history of adenoma or colon cancer	0.55

The influence of gender is attributed to significant differences between the genders for healthy controls, shown in Fig. 4A. At the same time, there are no significant gender differences for patients harboring advanced adenomas. Moreover, there remains a highly significant increase ($P < 0.001$) in the LEBS marker from female controls to females with advanced adenomas and from male controls to males with advanced adenomas. We furthermore

wanted to determine whether the LEBS marker was confounded by benign hyperplastic polyps. Figure 4B shows no significant changes in the LEBS marker relative to control for patients with hyperplastic polyps ($P = 0.66$).

Influence of advanced adenoma location

We next wanted to assess whether the rectal LEBS marker was sensitive to advanced adenomas found throughout the colon. We therefore separated patients with advanced adenomas into two groups according to the colon location in which the largest polyp was found. Proximal adenomas were defined as lesions found within the first portion of the colon before the splenic flexure (e.g., lesions in the cecum, ascending colon, hepatic flexure, or transverse colon), whereas distal adenomas were defined as lesions found in the final portion of the colon (e.g., lesions in the splenic flexure, descending colon, sigmoid colon, or rectum). Figure 4C shows a highly significant increase in the LEBS marker ($P < 0.001$) for all advanced adenomas, regardless of their position within the colon. There was no significant

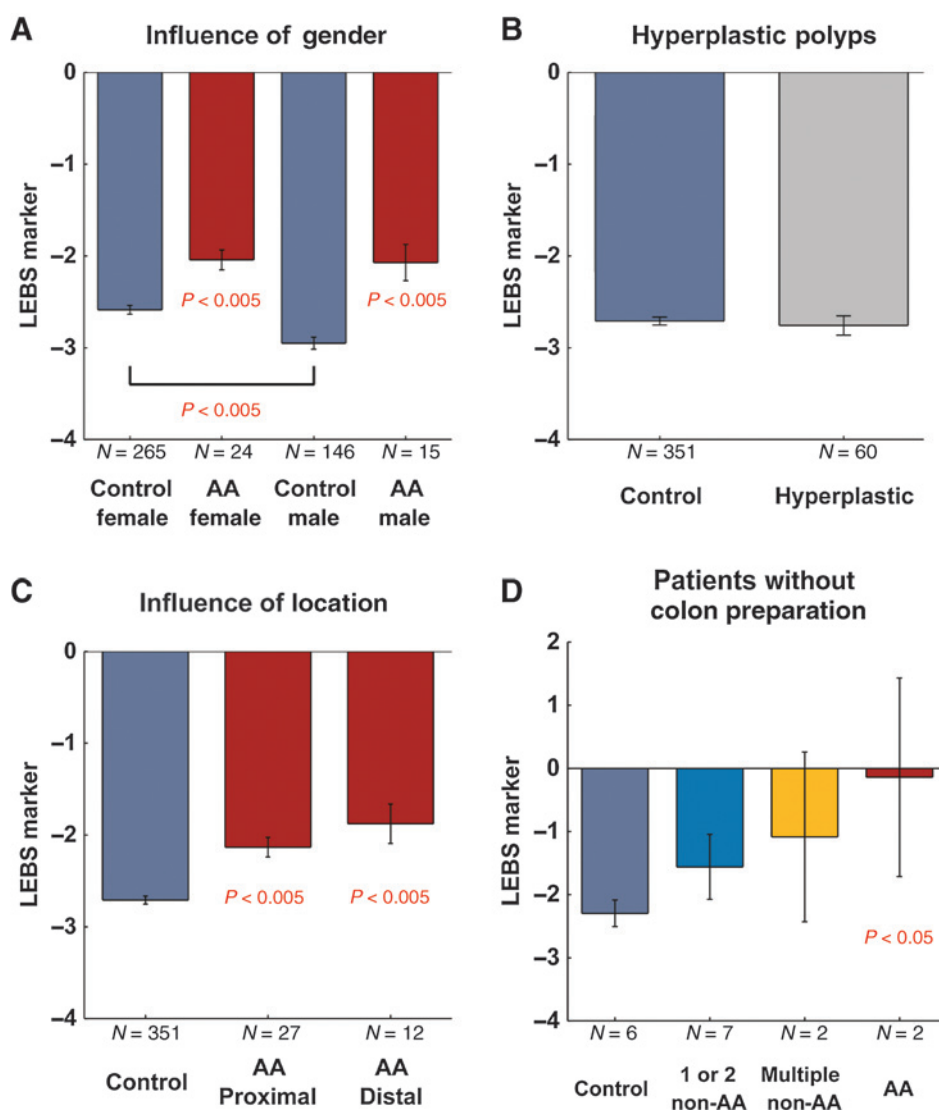


Figure 4. Diagnostic LEBS marker separated according to gender (A), other polyp types (B), and advanced adenoma location (C). D, diagnostic LEBS marker evaluated in patients without colonic preparation. In A, the P values displayed below the LEBS marker for male and female patients harboring advanced adenoma are both calculated with respect to the corresponding gender of healthy control.

difference in the LEBS marker between patients with proximal and distal advanced adenomas.

Pilot study of the LEBS marker in patients without colon preparation

Applying the model developed in the prepped training set to the smaller unprepped pilot study dataset shows a similar progressive increase in the LEBS marker for patients with increasing levels of dysplasia (shown in Fig. 4D). Relative to healthy controls, the LEBS marker is significantly increased ($P = 0.04$) for patients with advanced adenomas. At the same time, there are only insignificant differences measured for patients with non-advanced adenomas. Importantly, the values of the LEBS marker for healthy controls in this pilot study are consistent with both the training and validation datasets (i.e., there are nonsignificant differences in value). Furthermore, a paired t test on 7 patients who underwent LEBS measurement both with and without colon preparation showed insignificant differences between measurements ($P = 0.18$). Taken together, these results suggest that colon preparation may have a negligible effect on LEBS measurements. However, due to the limited sample size of this dataset, a larger study is needed to confirm these findings.

Discussion

In three previous studies of rectal biopsies (21–23), our group developed sophisticated optical instrumentations to establish the location and nature of the structural alterations occurring in colorectal cancer field carcinogenesis. The first study provided proof-of-principle for the diagnostic potential of rectal LEBS with an area under the ROC curve of 89.5% for patients harboring advanced adenomas (21). We next established that this diagnostic signal is the result of synergistic changes in the epithelial cells and surrounding stroma (i.e., $\downarrow\mu_s^*$ and $\uparrow D$ in both compartments; ref. 23). Armed with these observations *ex vivo*, we developed the minimally invasive LEBS probe to optimally interrogate the alterations *in vivo* (30).

In the current study, we confirmed that the trends observed *ex vivo* were recapitulated *in vivo*. First, we observed a significant decrease in μ_s^* and increase in D for patients harboring advanced adenoma. Importantly, the directionality of the changes was consistent with *ex vivo* findings (22). In order to form a single diagnostic marker, we combined μ_s^* and D in a logistic regression to formulate the LEBS marker. The cross-validated LEBS marker was highly sensitive to clinically relevant adenomas with 88% sensitivity for advanced adenomas, 71% sensitivity to multiple non-advanced adenomas, and 72% specificity in the validation set. Moreover, after incorporating a number of colorectal cancer risk factors (e.g., endoscopist, age, gender, race, smoking/alcohol/cancer history) into an ANCOVA model, the LEBS marker remained highly predictive of the presence of advanced adenoma ($P < 0.001$). Finally, we provided proof-of-principle for the operation of LEBS in patients without colon preparation.

From a mechanistic point of view, the changes in D provide fundamental insight into the changes in rectal mucosa morphology occurring in field carcinogenesis. Physically speaking, the observed increase in D indicates a shift in tissue ultrastructural composition toward larger features (i.e., small features aggregate to form larger structures). Although LEBS does not directly resolve the specific structures which contribute to this change, a number of different lines of evidence point to a synergistic combination of

intracellular and extracellular components (17, 23). Within the nucleus of histologically normal human rectal cells, we previously observed an upregulation in the histone deacetylase (HDAC) enzyme class responsible for enabling DNA to wrap more tightly around the histones (15). We subsequently confirmed that this change in gene expression resulted in an increase D using direct visual confirmation with transmission electron microscopy (18). In the extracellular tissue component, the increase in D is likely a result of collagen fiber bundling and cross-linking potentially induced by the upregulation of lysyl-oxidase or lysyl-oxidase like proteins (17, 23). One interesting observation about both of these structural changes is that they form a continuum with alterations typically associated with frank cancer. For example, chromatin compaction is a well-known marker of neoplasia that is commonly used in histopathology (38). However, although the changes in the later stages of cancer development occur at structural length-scales on the order of several microns, the changes in field carcinogenesis present themselves at ultrastructural length-scales on the order of tens to hundreds of nm.

From a clinical perspective, any viable colorectal cancer risk stratification method must be highly sensitive to premalignant advanced adenomas, support a high level of patient compliance, and unburden limited endoscopy capacity. With these goals in mind, the use of optical LEBS markers offers a number of attractive advantages for use in colorectal cancer pre-screening. First, is the high 88% sensitivity to advanced adenomas, which ensures that most patients who would benefit from colonoscopy screening are correctly identified. In comparison, other minimally invasive techniques such as fecal tests suffer from low test sensitivity to advanced adenomas [10.8%, 29.5%, and 42.4% sensitivity for FOBT (6, 7), FIT (7), and sDNA (4) respectively]—though overall program sensitivity is expected to increase when fecal tests are applied on an annual basis. Furthermore, the LEBS probe combines a design that is straightforward to implement and minimally intrusive to the patient. Such a design could therefore be implemented in the primary care setting in order to boost theoretical patient compliance. Finally, given the baseline performance and prevalence observed in the current study, the theoretical one-time use of LEBS prescreening would be more than half the number of colonoscopies needed to identify one advanced adenoma from 16 down to 7. Such an outcome would result in a more efficient use of endoscopy resources.

There are a number of important limitations in the current study that should be acknowledged. First, is the modest 72% specificity compared with FOBT (95.2%; ref. 6, 7), FIT (97.3%; ref. 7), and sDNA (86.6%; ref. 4)—meaning that a large number of unnecessary colonoscopies would still be performed under a theoretical LEBS prescreening program. Furthermore, we note that the rather high approximately 13% dropout rate for LEBS measurements based on rational marker exclusion criteria reduces the practical accuracy of a real-world LEBS test. With these two points in mind, we note that the current study presents data acquired with a first-generation *in vivo* LEBS instrument. Thus, the performance from this report should be viewed as the "floor" and underestimates the potential power of this approach in newer optimized design iterations.

Another limitation of the current study is that patients were enrolled through hospital locations with largely Caucasian demographics (83.4% in the current study). Although ANCOVA showed that the LEBS marker was not confounded by race, further clinical studies will need to draw from a more diverse

demographic pool in order to more fully validate the viability of our approach. Next, the modest number of advanced adenomas ($N = 41$) limits the precision with which test performance characteristics be determined. Furthermore, due to a non-zero colonoscopic miss rate ($\sim 1\%$ – 2% for advanced adenomas and $\sim 15\%$ – 25% for all adenomas; ref. 39), there is the potential for patient misclassification. Similarly, patient classification was carried out according to size estimates from colonoscopy that may over- or understate the actual size. Taken together, these limitations indicate that further clinical LEBS studies are still needed.

A further important limitation of the current study is that all patients in main study followed standard preparation protocols for colonoscopy, including colonic purge, before measurement with LEBS. To ensure optimal patient compliance, rectal LEBS analysis would need to be performed without the requirement of a colonic purge. Although we presented preliminary results demonstrating the potential of using rectal LEBS in patients without colon preparation, further studies are needed to fully assess the diagnostic potential of and patient compliance for such a procedure. Accordingly, the pilot unprepped should be viewed as a proof-of-principle for the operation of LEBS in patients without colonic purge.

In conclusion, we provide the first evidence that *in vivo* LEBS analysis of the rectal mucosa is sensitive to clinically relevant premalignant lesions throughout the colon. We envision the future use of LEBS as the first step in a two-tiered approach for colorectal cancer screening. Under this approach, patients within the general screening population would undergo LEBS analysis during their annual physical exam. Those patients deemed to be at the highest risk of developing colorectal cancer would then proceed to a follow-up colonoscopy in order to remove all precancerous adenomas via polypectomy. In order to move closer towards this goal, further studies of rectal LEBS applied to patients without colonic preparation are currently underway.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015;65:5–29.
2. Siegel RL, Ward EM, Jemal A. Trends in colorectal cancer incidence rates in the United States by tumor location and stage, 1992–2008. *Cancer Epidemiol Biomarkers Prev* 2012;21:411–6.
3. Levin B, Lieberman DA, McFarland B, Smith RA, Brooks D, Andrews KS, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *CA Cancer J Clin* 2008;58:130–60.
4. Imperiale TF, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, et al. Multitarget stool DNA testing for colorectal-cancer screening. *N Engl J Med* 2014;370:1287–97.
5. Winawer S, Fletcher R, Rex D, Bond J, Burt R, Ferrucci J, et al. Colorectal cancer screening and surveillance: clinical guidelines and rationale—Update based on new evidence. *Gastroenterology* 2003;124:544–60.
6. Imperiale TF, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004;351:2704–14.
7. Allison JE, Sakoda LC, Levin TR, Tucker JP, Tekawa IS, Cuff T, et al. Screening for colorectal neoplasms with new fecal occult blood tests: update on performance characteristics. *J Natl Cancer Inst* 2007;99:1462–70.
8. Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. *Cancer* 1953;6:963–8.
9. Shen L, Kondo Y, Rosner GL, Xiao L, Hernandez NS, Vilaythong J, et al. MGMT promoter methylation and field defect in sporadic colorectal cancer. *J Natl Cancer Inst* 2005;97:1330–8.
10. Franklin WA, Gazdar AF, Haney J, Wistuba II, La Rosa FG, Kennedy T, et al. Widely dispersed p53 mutation in respiratory epithelium. A novel mechanism for field carcinogenesis. *J Clin Invest* 1997;100:2133–7.
11. Mehrotra J, Varde S, Wang H, Chiu H, Vargo J, Gray K, et al. Quantitative, spatial resolution of the epigenetic field effect in prostate cancer. *Prostate* 2008;68:152–60.
12. Prevo LJ, Sanchez CA, Galipeau PC, Reid BJ. p53-mutant clones and field effects in Barrett's esophagus. *Cancer Res* 1999;59:4784–7.
13. Braakhuis BJ, Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. *Cancer Res* 2003;63:1727–30.
14. Dakubo GD, Jakupciak JP, Birch-Machin MA, Parr RL. Clinical implications and utility of field cancerization. *Cancer Cell Int* 2007;7:2.
15. Stypula-Cyrus Y, Damanian D, Kunte DP, Cruz MD, Subramanian H, Roy HK, et al. HDAC up-regulation in early colon field carcinogenesis is involved in cell tumorigenicity through regulation of chromatin structure. *PLoS ONE* 2013;8:e64600.
16. Stypula-Cyrus Y, Mutyal NN, Dela Cruz M, Kunte DP, Radosevich AJ, Wali R, et al. End-binding protein 1 (EB1) up-regulation is an early event in colorectal carcinogenesis. *FEBS Lett* 2014;588:829–35.
17. Backman V, Roy HK. Advances in biophotonics detection of field carcinogenesis for colon cancer risk stratification. *J Cancer* 2013;4:251–61.

Disclosure of Potential Conflicts of Interest

B. Gould is an employee of and has ownership interest (including patents) in American BioOptics, LLC. M.J. Goldberg has ownership interest (including patents) in American BioOptics, LLC, and Nanocytomic. V. Backman and H.K. Roy have ownership interests (including patents) in American BioOptics, LLC. No potential conflicts of interest were disclosed by the other authors.

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18. Cherkezyan L, Stypula-Cyrus Y, Subramanian H, White C, Dela Cruz M, Wali RK, et al. Nanoscale changes in chromatin organization represent the initial steps of tumorigenesis: a transmission electron microscopy study. *BMC Cancer* 2014;14:189.
19. Lewis JD, Ng K, Hung KE, Bilker WB, Berlin JA, Brensinger C, et al. Detection of proximal adenomatous polyps with screening sigmoidoscopy: a systematic review and meta-analysis of screening colonoscopy. *Arch Intern Med* 2003;163:413–20.
20. Roy HK, Turzhitsky V, Gomes A, Goldberg MJ, Rogers JD, Kim YL, et al. Prediction of colonic neoplasia through spectral marker analysis from the endoscopically normal rectum: an *ex vivo* and *in vivo* study. *Gastroenterology* 2008;134:A109-A.
21. Roy HK, Turzhitsky V, Kim Y, Goldberg MJ, Watson P, Rogers JD, et al. Association between rectal optical signatures and colonic neoplasia: potential applications for screening. *Cancer Res* 2009;69:4476–83.
22. Radosevich AJ, Mutyal NN, Yi J, Stypula-Cyrus Y, Rogers JD, Goldberg MJ, et al. Ultrastructural alterations in field carcinogenesis measured by enhanced backscattering spectroscopy. *J Biomed Opt* 2013;18.
23. Yi J, Radosevich AJ, Stypula-Cyrus Y, Mutyal NN, Azarin SM, Horcher E, et al. Spatially resolved optical and ultrastructural properties of colorectal and pancreatic field carcinogenesis observed by inverse spectroscopic optical coherence tomography. *J Biomed Opt* 2014;19:36013.
24. Subramanian H, Roy HK, Pradhan P, Goldberg MJ, Muldoon J, Brand RE, et al. Nanoscale cellular changes in field carcinogenesis detected by partial wave spectroscopy. *Cancer Res* 2009;69:5357–63.
25. Gomes AJ, Roy HK, Turzhitsky V, Kim Y, Rogers JD, Ruderman S, et al. Rectal mucosal microvascular blood supply increase is associated with colonic neoplasia. *Clin Cancer Res* 2009;15:3110–7.
26. Turzhitsky VM, Gomes AJ, Kim YL, Liu Y, Kromine A, Rogers JD, et al. Measuring mucosal blood supply *in vivo* with a polarization-gating probe. *Appl Opt* 2008;47:6046–57.
27. Kim YL, Liu Y, Wali RK, Roy HK, Backman V. Low-coherent backscattering spectroscopy for tissue characterization. *Appl Opt* 2005;44:366–77.
28. Radosevich AJ, Yi J, Rogers JD, Backman V. Structural length-scale sensitivities of reflectance measurements in continuous random media under the Born approximation. *Opt Lett* 2012;37:5220–2.
29. Rogers JD, Radosevich AJ, Ji Y, Backman V. Modeling light scattering in tissue as continuous random media using a versatile refractive index correlation function. *Selected Topics in Quantum Electronics, IEEE Journal of* 2014;20:1–14.
30. Mutyal NN, Radosevich A, Gould B, Rogers JD, Gomes A, Turzhitsky V, et al. A fiber optic probe design to measure depth-limited optical properties *in vivo* with low-coherence enhanced backscattering (LEBS) spectroscopy. *Opt Express* 2012;20:19643–57.
31. Rogers JD, Stoyneva V, Turzhitsky V, Mutyal NN, Pradhan P, Capoglu IR, et al. Alternate formulation of enhanced backscattering as phase conjugation and diffraction: derivation and experimental observation. *Opt Exp* 2011;19:11922–31.
32. Turzhitsky V, Mutyal NN, Radosevich AJ, Backman V. Multiple scattering model for the penetration depth of low-coherence enhanced backscattering. *J Biomed Opt* 2011;16:097006.
33. Radosevich AJ, Rogers JD, Turzhitsky V, Mutyal NN, Yi J, Roy HK, et al. Polarized enhanced backscattering spectroscopy for characterization of biological tissues at subdiffusion length scales. *IEEE J Sel Top Quant* 2012;18:1313–25.
34. Rogers JD, Capoglu IR, Backman V. Nonscalar elastic light scattering from continuous random media in the Born approximation. *Opt Lett* 2009;34:1891–3.
35. Turzhitsky V, Radosevich AJ, Rogers JD, Mutyal NN, Backman V. Measurement of optical scattering properties with low-coherence enhanced backscattering spectroscopy. *J Biomed Opt* 2011;16:067007.
36. Cheong WF, Pahl SA, Welch AJ. A review of the optical-properties of biological tissues. *IEEE J Quantum Elect* 1990;26:2166–85.
37. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982;143:29–36.
38. Zink D, Fischer AH, Nickerson JA. Nuclear structure in cancer cells. *Nat Rev Cancer* 2004;4:677–87.
39. van Rijn JC, Reitsma JB, Stoker J, Bossuyt PM, van Deventer SJ, Dekker E. Polyp miss rate determined by tandem colonoscopy: a systematic review. *Am J Gastroenterol* 2006;101:343–50.

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