Research Article

Cancer Prevention Research

Optical Measurement of Rectal Microvasculature as an Adjunct to Flexible Sigmoidosocopy: Gender-Specific Implications

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Abstract

Flexible sigmoidoscopy is a robust, clinically validated, and widely available colorectal cancer screening technique that is currently sanctioned by major guideline organizations. Given that endoscopic visualization is generally limited to the distal third of the colon and women tend to have a proclivity for proximal lesions, the flexible sigmoidoscopy performance is markedly inferior in women than in men. Our group has shown that by using a novel light-scattering approach, we were able to detect an early increase in blood supply (EIBS) in the distal colonic mucosa, which served as a marker of field carcinogenesis and, hence, proximal neoplasia. Therefore, we sought to ascertain whether rectal EIBS would improve flexible sigmoidoscopy, especially in women. A polarization-gated spectroscopy fiber-optic probe was used to assess EIBS in the endoscopically normal rectum (n = 366). When compared with gender-matched neoplasia-free controls, females with advanced proximal neoplasia (n = 10) had a robust (60%; P = 0.002) increase in rectal mucosal oxyhemoglobin content whereas the effect size in males was less marked (33%; P = 0.052). In women, addition of rectal oxyhemoglobin tripled the sensitivity for advanced neoplasia over flexible sigmoidoscopy alone. Indeed, the performance characteristics seemed to be excellent (sensitivity, 100%; specificity, 76.8%; positive predictive value, 32.6%; and negative predictive value, 100%). A variety of nonneoplastic factors were assessed and did not confound the relationship between rectal EIBS and advanced neoplasia. Therefore, using rectal EIBS in combination with flexible sigmoidoscopy mitigated the gender gap and may allow flexible sigmoidoscopy to be considered as a viable colorectal cancer screening test in women. *Cancer Prev Res; 3(7); 844–51. ©2010 AACR.*

Introduction

Colorectal cancer (CRC) remains the third leading cause of death in both women and men, underscoring the need for more effective prevention strategies in both genders (1). Although the lifetime incidence of CRC is roughly equivalent between women and men (5.1% versus 5.5%, respectively), there are some important distinctions (1). Biologically, lesions in women tend to have a more proximal neoplasia and a higher incidence of microsatellite instability (2). Stage for stage, women with CRC have a better prognosis than men (3). From a genetic/epigenetic perspective, it has been noted that specific polymorphisms can be associated with opposite effects on survival in men and women. There is also evidence that some CRC risk factors may also have gender-specific manifestations (4). This gender predilection seems to be mirrored in exogenous CRC risk factors such as obesity or tobacco use (5, 6). The biological basis for these gender-specific effects is incompletely elucidated, although much attention has centered on sex hormones given the well-established chemopreventive efficacy of estrogen/progesterone (7) and the observation that estrogen receptor β functions as a tumor suppressor gene (8–10).

There has been emerging interest in understanding the implications of gender on CRC screening strategies. One of the most salient consequences of gender on colon carcinogenesis is the predilection toward proximal neoplasia in women (6). In a landmark report, Schoenfeld and colleagues noted that whereas flexible sigmoidoscopy detected two thirds of advanced neoplasia in men, it only detected one third in women (11). These concerns over performance, especially in women, have meant that flexible sigmoidoscopy has by and large been eschewed for CRC screening among Americans. However, flexible sigmoidoscopy has numerous attractive features including cost, availability (performed by primary care providers),

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and patient acceptability, underscoring the need to improve this test. Indeed, flexible sigmoidoscopy remains one of only two tests currently recommended by both major guidelines for colorectal adenoma/carcinoma screening (12, 13).

Flexible sigmoidoscopy can provide some insight into the risk of proximal neoplasia via identification of field carcinogenesis. Field carcinogenesis (also known as field cancerization, field effect, field defect, etc.) is the concept that the genetic/environmental milieu that leads to a focal neoplastic lesion in one area of the colon should be detectable, at least in some form, throughout the colon (4, 14). Indeed, standard clinical practice mandates a full colonoscopy if an adenoma (acting as a marker of the field effect) is detected in the distal colon (13). Unfortunately, as a marker of proximal neoplasia, the distal adenoma is quite insensitive, missing more than half of advanced proximal neoplasia (15, 16). Therefore, attention has been focused on identifying earlier events in carcinogenesis such as focal morphologic lesions (aberrant crypt foci; ref. 17) or cellular markers (apoptosis/proliferation; refs. 18, 19) in the histologically normal distal colonic mucosa. Although all these lesions correlate with proximal neoplasia, their performance characteristics are inadequate. Clearly, there is an urgent need to develop novel biomarkers from the distal colon that would be sensitive to proximal colonic lesions, thus allowing a resurgence of flexible sigmoidoscopy as a viable CRC screening technique, especially in women.

Our multidisciplinary CRC group has been focused on using advanced light-scattering technologies to detect colonic field carcinogenesis. Using an optical technology called four-dimensional elastically scattered-light fingerprinting (20), we observed that the pericryptal microvascular blood content is elevated before occurrence of neoplasia in two experimental models of colon carcinogenesis, the azoxymethane-treated rat and the MIN (multiple intestinal neoplasia) mouse (21). We recently reported in a study of 222 patients with a novel polarization-gated spectroscopy fiber-optic probe that the microvascular blood content was elevated in the endoscopically normal mucosae of patients harboring neoplasia (22). This phenomenon, termed early increase in blood supply (EIBS), was noted throughout the colon. Importantly, the endoscopically normal rectal mucosa manifested elevated microvascular blood content in patients harboring advanced neoplasia, even in the proximal colon (23). We therefore hypothesized that EIBS measurements may provide a powerful adjunct to flexible sigmoidoscopy, especially in women for whom flexible sigmoidoscopy alone performs particularly poorly.

Materials and Methods

Participants and acquisition of clinical data

All studies were approved and conducted under the supervision of the Institutional Review Board at NorthShore University HealthSystem (previously known as Evanston-Northwestern Healthcare). Patients undergoing colonoscopy for screening or surveillance were included in the study and these patients were unselected to replicate a typical screening population (age >50 years or a younger age coupled with family history of CRC). Exclusion criteria included inability to give informed consent, colitis, poor preparation, and failure to intubate the cecum (because the presence of a proximal adenoma would not be detected). This study is composed of a total of 366 patients. At the conclusion of the withdrawal (visualization) phase for colonoscopy, the fiber-optic probe was inserted into the accessory channel of the colonoscope and the probe tip was placed in gentle contact with the rectal surface. The rectum was chosen as an ideal site for measurements because it is easily accessible and all flexible sigmoidoscopies invariably have access to the rectum despite any technical difficulties. On average, nine endoscopically normal rectal sites were selected for probe measurements, each requiring ~50 ms. The per-patient rectal hemoglobin content was determined as the average of these individual rectal readings. All polyps found during the procedure were sent for pathologic analysis and classified as benign lesions (i.e., hyperplastic polyps), nonadvanced adenomas, or advanced adenomas. Advanced adenomas were defined as adenomas with size ≥10 mm, >25% villous component, or having presence of high-grade dysplasia.

Endoscopically compatible fiber-optic probe for measurement of microvascular blood supply

The probe design has been described in detail in recent publications (22). Briefly, we developed a polarization-gated fiber-optic probe compatible with the accessory channel of a standard or pediatric colonoscope as shown in Fig. 1A. The probe enables measurement of the concentration of oxygenated and deoxygenated blood hemoglobin within the first 95 μm into tissue, which roughly corresponds to the depth of the mucosa. For the first 216 patients enrolled in the study, the clinical setup used a 75-W arc lamp and an integrated charge-coupled device spectrometer described in our previous studies. For the subsequent 150 patients, we simplified the instrumentation to minimize cost and size for greater clinical applicability. This simplified clinical setup includes a white-light LED (WT&T) that served as an illumination source and two fiber-optic spectrometers (Ocean Optics) for detection of light from the tissue. Data processed from the spectrometers were analyzed and displayed in real time using a laptop computer. Hemoglobin content was extracted from the tissue signals using the algorithm described in a previous publication (22). The compact EIBS instrument is illustrated in Fig. 1B.

Statistical analysis

Statistical analysis was done using Stata 9 (StataCorp) and Microsoft Excel. Markers were compared between no-neoplasia patients and advanced neoplasia patients using a two-sided Welch's t test. A two-sided P value <0.05 was considered statistically significant. The influence of

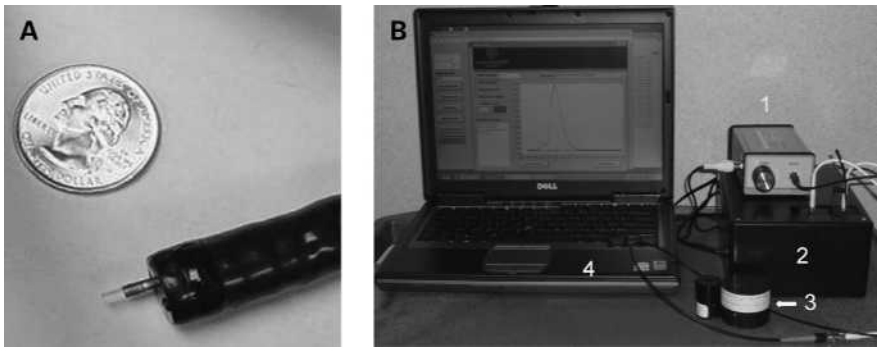

Fig. 1. Fiber-optic polarization-gated probe and clinical data collection system for real-time measurements *in vivo*. A, the 2.45-mm-diameter probe protruding from an accessory channel of an endoscope. B, photograph of the compact EIBS instrument with real-time capability for EIBS detection consisting of white-light LED light source (1), fiber-optic spectrometers (2), calibration stage (3), and laptop for real-time data display and analysis (4).

demographic factors was found using a least squares multivariate linear regression.

Diagnostic performance for discrimination between patients without neoplasia and those with advanced neoplasia was assessed with logistic regression using a single marker (mucosal oxygenated Hb content). A receiver operating characteristic (ROC) curve was generated by plotting sensitivity and 1 – specificity for the whole range of possible cutoff values. The area under the ROC curve represents the probability that the logistic regression model will give a higher predicted probability to a randomly selected positive sample versus a randomly chosen negative sample. This metric is used to summarize the overall accuracy of the logistic regression algorithm. To test the stability of the logistic regression model, a leave-one-out cross-validation was performed using MATLAB. Leave-one-out cross-validation trains the algorithm on all samples except for one point that serves as the test set. The process is repeated until all samples have been tested. For each iteration, a logistic model was formed using marker values from the $n - 1$ training samples and a predicted probability was calculated for the test point. After all iterations were completed, another ROC curve was generated using the predicted probabilities determined by the leave-one-out cross-validation algorithm.

Results

Patient characteristics

We obtained readings from 366 patients undergoing colonoscopy. Of these 366 patients, 327 were Caucasian and 17 had polyps identified and removed during previous colonoscopy. Grouped by current pathologic findings, 271 patients were adenoma-free, 67 had nonadvanced adenomas, and 28 patients with advanced neoplasia detected (defined as an advanced adenoma or colonic malignancy). After grouping by gender and location of the advanced neoplasia (distal colon was defined as rectum to splenic flexure whereas proximal colon included transverse, ascending and cecum), there were 10 female patients with advanced proximal neoplasia and 6 male patients with advanced proximal neoplasia. In addition, there were 4 female patients with advanced distal neoplasia and 8 male patients with advanced distal neoplasia. Subjects with no neoplasia had a mean age of 57 ± 12 years and 124 (45%) were females. The subjects with adenomas had a mean age of 58 ± 10 years and 22 (33%) were females, and the subjects with advanced neoplasia had a mean age of 61 ± 12 years and 14 (50%) were females. Table 1 summarizes the demographics for female patients with and without neoplasia. Advanced neoplasia patients had a significantly greater proportion of patients >55 years of age when compared with patients without neoplasia (P value = 0.048).

Assessment of EIBS markers

We analyzed rectal mucosal hemoglobin content from patients belonging to two categories: patients without neoplasia and patients with advanced neoplasia. We showed in previous studies that the penetration depth of ~95 μm elicited the greatest diagnostic separation between patients with and without neoplasia (22). We therefore focused only on this depth in our analysis. In Fig. 2A and B, we have compared the oxyhemoglobin (OHb) and deoxyhemoglobin

Table 1. *P* values for test of two proportion analyses showing differences in demographic factors between the no-neoplasia and disease groups

Demographic	Adenoma	Advanced adenoma
Age (<55 vs ≥55 y)	N.S.	0.048
Race (white vs nonwhite)	N.S.	N.S.
Current alcohol status	N.S.	N.S.
Current smoking status	N.S.	N.S.
Personal history of polyp	N.S.	N.S.
Personal history of CRC	N.S.	N.S.
Family history of CRC	N.S.	N.S.

Abbreviation: N.S., not significant ($P > 0.05$).

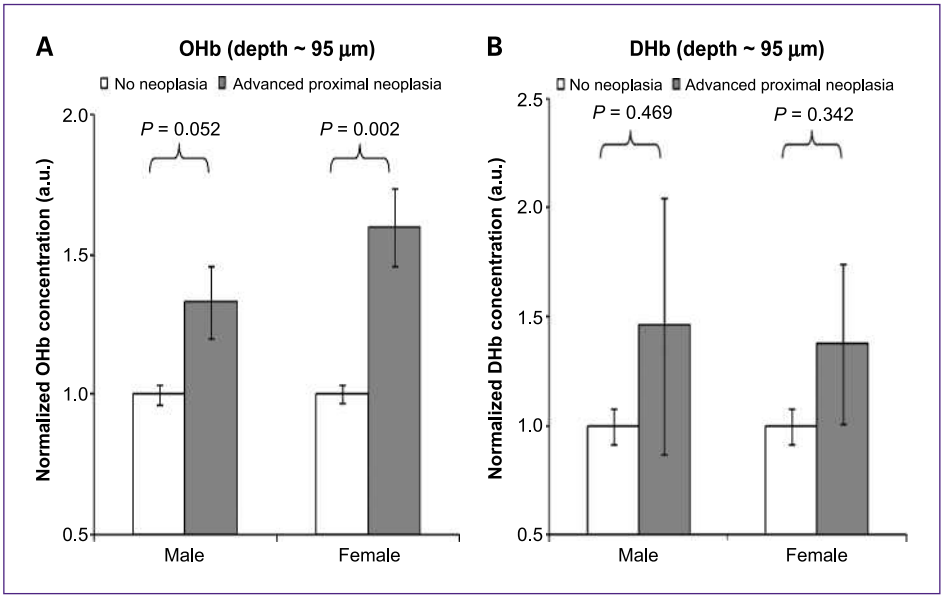

Fig. 2. Rectal OHb content from a tissue depth of ~95 μm is significantly elevated in women with advanced proximal neoplasia. A, OHb content was elevated for both men (n = 6) and women (n = 10) with advanced proximal neoplasia but the effect was more pronounced in women (effect size, 60%; P = 0.002). B, DHb content was also elevated in men and women with advanced proximal neoplasia but the effect size was not significant. Bars, SE.

(DHb) content for patients without neoplasia and those with advanced proximal neoplasia after grouping patients by gender. Figure 2 illustrates two key findings: First, by comparing A and B, we observed that the effect size was statistically significant for OHb but not for DHb. Second, Fig. 2A shows that female patients with advanced neoplasia in the proximal colon give rise to a highly significant ~60% increase in rectal OHb content (P = 0.002), whereas the effect is muted in male patients (effect size, 33%; P = 0.052). For female patients without neoplasia, demographic factors did not influence rectal OHb content, as shown in Table 2. These results suggest that rectal OHb content could be used as an adjuvant to flexible sigmoidoscopy to improve detection of proximal neoplasia in women.

Performance characteristics

After determining from Fig. 2 that rectal OHb was a promising marker for proximal neoplasia in women, we next investigated the diagnostic performance of this marker for advanced neoplasia both in the case of advanced proximal lesions only and in conjunction with flexible sigmoidoscopy for advanced lesions located anywhere in the colon. The area under the ROC curve using rectal OHb alone to predict female patients with advanced proximal neoplasia was 0.88, as shown in Fig. 3. Using the 100% sensitivity cutoff from this ROC curve yields 100% sensitivity, 76.8% specificity, 25.6% positive predictive value (PPV), and 100% negative predictive value (NPV). The sensitivity and NPV performance characteristics compare favorably with performance characteristics for a hypothetical flexible sigmoidoscopy calculated using our data set, assuming that any distal adenoma finding would trigger a full colonoscopy (0% sensitivity, 91.9% specificity, 0% PPV, and 92.6% NPV). These values are summarized and compared in Table 3. The sensitivity of rectal EIBS also compares favorably with the <10% sensitivity for flexible sigmoidoscopy noted in the literature (3). After leave-one-out cross validation, the ROC curve remained high at 0.86, indicating a robust classification. We also point out an extremely low probability of data overfitting because only one marker (OHb) was used in this study.

We next investigated how combining distal endoscopic findings and rectal OHb content could predict which female patients would have advanced neoplasia anywhere in the colon. This analysis simulates the clinical application of performing flexible sigmoidoscopy along with rectal blood content measurements to screen the entire colon for advanced neoplasia. The distal findings from the colonoscopy served to replicate the hypothetical findings of a flexible sigmoidoscopy procedure. In addition, for the combined test, we used only the rectal OHb findings to

Table 2. Multivariate linear regression ANOVA P values showing the effects of demographic factors on measured OHb in women (depth ~95 μm)

Demographic	Adenoma P values
Age (<55 vs ≥55 y)	0.222
Race (white vs nonwhite)	0.460
Current alcohol status	0.440
Current smoking status	0.850
Personal history of polyp	0.950
Personal history of CRC	0.600
Family history of CRC	0.550

NOTE: Only no-neoplasia patients (n = 124) were used in the analysis.

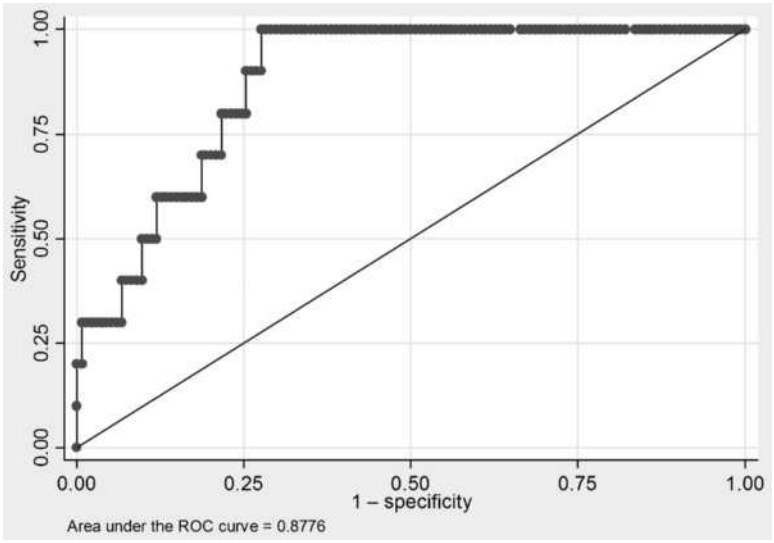

Fig. 3. ROC curve showing that measurement of rectal OHb content allows accurate prediction of advanced proximal neoplasia in women. The area under the curve is 0.88, allowing 100% sensitivity and 76.8% specificity to be obtained.

predict proximal lesions and we used only the distal endoscopic findings to predict the distal lesions. Because the distal endoscopic findings detect all advanced distal neoplasia, the performance characteristics of the combined test closely mirror the performance characteristics for the detection of advanced proximal neoplasia using rectal OHb content. The performance characteristics for the combined test in predicting advanced neoplasia anywhere in the colon were 100% sensitivity, 76.8% specificity, 32.6% PPV, and 100% NPV. This significantly improves the performance of flexible sigmoidoscopy alone, which had the following performance for neoplasia anywhere in the colon: 28.6% sensitivity, 94.0% specificity, 33.3% PPV, and 92.6% NPV.

Discussion

We show herein that rectal mucosal microvascular blood content assessment via polarization-gated spectroscopy allowed highly accurate discrimination of patients harboring advanced proximal neoplasia from those who were neoplasia-free. Interestingly, the effect size of rectal EIBS seemed to be greater in women than in men. This is particularly significant because we confirmed that flexible sigmoidoscopy per se was markedly inferior in women. Importantly, the combination of rectal EIBS with flexible sigmoidoscopy leads to excellent performance characteristics in both genders. Thus, using rectal EIBS as a biomarker was able to mitigate the gender disparities that are inherent in CRC screening with flexible sigmoidoscopy.

Table 3. Statistical performance of predicting advanced proximal neoplasia in women using flexible sigmoidoscopy versus rectal OHb content

Performance characteristic	Flexible sigmoidoscopy only (%)	Rectal OHb content (%)
Sensitivity	0	100
Specificity	94.3	76.8
PPV	0	25.6
NPV	92.6	100

There is emerging evidence that gender can significantly affect colon carcinogenesis. For instance, in a large-scale prospective randomized trial (the Women's Health Initiative), supplementation with estrogen/progesterone was noted to cause a marked (38%) reduction in CRCs (7). Epidemiologically, women tend to have later onset of disease (6). Biologically, the lesions tend to be more right sided, consonant with an increased prevalence of microsatellite instability (2). There has been the recent suggestion that a higher proportion of adenomas may progress to carcinomas in women versus men (24). At a molecular level, there are a variety of genes (*p53*, *phosphatidylinositol 3-kinase*, etc.) that are differentially expressed in colon cancers from women when compared with men (10, 25). Moreover, genetic/epigenetic alterations may be gender-specific consequences, as shown by a report that epidermal growth factor receptor polymorphisms had opposite effects on CRC survival in men and women (4). Finally, from a risk factor perspective, gender has been shown to alter the clinical manifestations of other risk CRC factors such as body mass index or tobacco smoking (5, 6).

Despite the increasing appreciation of differences between women and men in CRC biology, the most recent

screening guidelines have remained gender-neutral. The recommended screening options range from the minimally invasive but poorly sensitive (e.g., fecal detection of hemoglobin or DNA mutations) to the more invasive and sensitive (e.g., colonoscopy). The fecal tests are able to detect carcinomas insensitive to adenomas. Because the major goal of screening is cancer prevention through interruption of the adenoma-carcinoma progression, the multigroup consortium (including American Cancer Society, American Gastroenterological Association, and American College of Radiology) has somewhat relegated fecal tests to second line in favor of techniques that may detect adenomas (13). Only colonoscopy and flexible sigmoidoscopy are recommended by both the multigroup consortium and the U.S. Preventive Service Task Force for CRC prevention. Colonoscopy is considered by many authorities to be the gold standard. It has high accuracy, examines the entire colonic mucosa, and allows polyp removal in the same session (thus leading to cancer prevention). However, it is expensive, invasive, may be uncomfortable, and carries significant risks. Furthermore, the resources required (endoscopic capacity and cost) for screening the entire average risk population (>90 million Americans over age 50) seem to be prohibitive (14). With regard to gender-specific issues, the ability for colonoscopy to prevent against proximal CRCs has recently been questioned (26), which is concerning for women because of the predilection for right-sided lesions (6, 11). Indeed, a recent study indicated that women had a higher risk of developing CRC after colonoscopy (4.1% versus 2.9% for men, $P < 0.001$; ref. 27). Additionally, women tend to have longer colons in smaller abdominal cavity and more likely to have had prior abdominal/pelvic surgeries, making colonoscopy more painful (28). Thus, using colonoscopy as the sole option for average-risk population screening, especially in women, may not be optimal.

Flexible sigmoidoscopy has been a stalwart of CRC screening for many decades and is sanctioned by major guideline committees for cancer prevention. Flexible sigmoidoscopy has several advantages over colonoscopy, including a more tolerable bowel purge (a leading obstacle to undergo colonoscopy or CTC) and no need for sedation because of less discomfort. Indeed, patients' preferences for flexible sigmoidoscopy seem to be equivalent to colonoscopy despite the latter's superior efficacy (29). Furthermore, unlike colonoscopy, flexible sigmoidoscopy can be done by primary care physicians or even mid-level staff (nurse practitioners and physician's assistants), thus being well suited for population screening. The efficacy of flexible sigmoidoscopy in CRC prevention is well established, with both case-control and prospective studies indicating a 80% to 90% risk reduction in the distal colon (30–32), although one recent study failed to show any protection over 7 years (33).

The limitations of flexible sigmoidoscopy relate to the inability to directly assess proximal neoplasia, which is particularly concerning for in women given the predilection for right-sided CRCs. Some insight into the presence of proximal neoplasia can be gleaned from identifying field carcinogenesis, but unfortunately, the sentinel adenoma is noted in less than half the patients with advanced proximal neoplasia (16). Data from our study confirm this finding, showing a much lower rate in women than in men (28.6% versus 57.1%). This is consistent with emerging data that even advanced adenomas may be a less predictive marker of CRC in women (24). There are other precedents for biomarker accuracy for colon carcinogenesis that are different between men and women, such as the C-reactive protein (34). Thus, not only are better biomarkers as adjuncts to flexible sigmoidoscopy urgently needed but the gender-specific performance also needs to be carefully determined.

This work builds on our initial report from a different (smaller) cohort that rectal EIBS was elevated in patients harboring advanced adenomas elsewhere in their colons (23). We have previously reported that four-dimensional elastically scattered-light fingerprinting was able to detect various facets of EIBS (OHb, DHb, and packaging length scale) and we corroborated this with immunoblot analysis of mucosal hemoglobin (21, 22). This phenomenon was depth selective, with the maximum diagnostic information at ~95 μm (which was used for these studies). Moreover, for simplicity and to avoid overfitting, we used a single marker (OHb) in our current study. Additionally, whereas our previous report had shown a comparable effect size for advanced adenomas regardless of location, in this study, we focused exclusively on proximal lesions based on the potential clinical application (23). Detection of distal lesions by rectal EIBS is moot because in the adjunct to flexible sigmoidoscopy, these would have been endoscopically visualized. We chose advanced adenomas because these are the best established intermediate lesions in screening studies (fecal DNA, CT colography, etc.; refs. 35, 36). Our previous study had shown that rectal EIBS was not sensitive to smaller lesions (23). Fortunately, these smaller adenomas are generally of no clinical consequence because they rarely harbor advanced features and their natural history is either slow growth or actual regression (37). The novel aspect of our current study is the gender-related accentuation of rectal EIBS and the coupling of this biomarker to flexible sigmoidoscopy. Importantly, we noted that the increased performance of EIBS in women seemed to effectively mitigate the poorer gender-specific performance of flexible sigmoidoscopy (11).

The biological basis for the greater sensitivity of rectal EIBS in women was not explored in this study. There are potentially a myriad of factors involved. For instance, inducible nitric oxide synthase seems to be logical, given our data on the pivotal role of nitric oxide in the pathogenesis of EIBS in the azoxymethane-treated rat model (38). An intriguing report indicated that female rats had greater mesenteric nitric oxide production than males, suggesting potential biological underpinnings for the gender-specific inducible nitric oxide synthase-EIBS relationship (39). Alternatively, estrogens have been shown

to affect microcirculation (coronary) directly or through alterations in RBC deformity (40). Because most women who develop CRC are postmenopausal (hence minimal ovarian estrogen secretion), we need to be circumspect about the speculations on the role of sex hormones in EIBS. However, polyp development takes a number of years, and it is probable that ovarian steroids contributed during the initiation phases. Gender may also affect other mediators of EIBS. For instance, the fact that smoking may independently alter EIBS (22) is particularly apropos given our report that women are more susceptible to the pro-colon carcinogenic effect of cigarettes (6). Further supporting the gender-colonic blood supply relationship is the observation that vascular endothelial growth factor (*VEGF*) polymorphisms had differential effects on CRC risk in men and women (41). It needs to be emphasized that these mechanistic possibilities are simply speculations and require experimental confirmation.

There are a number of limitations in the study that need to be addressed. The first is that despite the significant number of patients recruited for this study ($n = 366$), there is a relatively small number of advanced proximal neoplasms in the female subgroup ($n = 10$) that is consistent with an average-risk screening population. It is encouraging that the effect size was sufficient to achieve statistical significance. Although overfitting is possible, these concerns are mitigated by the use of a single biomarker (OHb). We plan to conduct a larger prospective validation trial to unequivocally confirm these findings. This report does not address the biological basis for gender differences in EIBS, with the possibilities discussed above being speculations and requiring experimental confirmation. On the other hand, irrespective of mechanisms, this study underscores the need to evaluate CRC biomarkers such as EIBS in a gender-specific context. Another issue is inaccuracy in endoscopic adenoma size assessment (>10 mm), although this would not bias our results (42). Finally, we used colonoscopic examination of the distal colon to replicate flexible sigmoidoscopy. This is not entirely representative of the delivery of flexible sigmoidoscopy in the community where the endoscope is typically not inserted to the splenic flexure, secondary to less experienced endoscopists (primary care physicians), poorer bowel purge, and lack of sedation. Moreover, in women, the depth of insertion is often significantly less than that in men (43). Although not really effecting our results presented herein, we plan to conduct prospective studies that will use more "real-life" flexible sigmoidoscopy conditions.

In conclusion, we showed that rectal microvascular blood content was elevated in patients harboring advanced proximal neoplasia with an effect size and hence performance characteristics superior in women than in men. This is of considerable importance given that flexible sigmoidoscopy is inferior in women largely due to proximal distribution of adenomas. It is striking to note that when flexible sigmoidoscopy and rectal EIBS were combined, the performance characteristics in both women and men were excellent. If validated in future studies, we envision that during a conventional flexible sigmoidoscopy, the endoscopist could also take several rectal EIBS readings (generally taking ~50 ms per reading). If either the flexible sigmoidoscopy (presence or distal adenoma) or rectal EIBS is positive, the patient would be referred for full colonoscopy. Further studies will be needed to validate this finding. Moreover, this work highlights the need to be cognizant of gender-selective effects when developing biomarkers for cancer screening.

Disclosure of Potential Conflicts of Interest

H.K. Roy, M.J. Goldberg, and V. Backman are cofounders and shareholders of American BioOptics LLC. American BioOptics LLC had no role in the design or execution of the study, data analysis, or manuscript preparation. All aspects of the study and manuscript preparation were done under the supervision of the conflict of interest committee at Northwestern University. The other authors disclosed no potential conflicts of interest.

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