Diagnostic Challenges Caused by Endoscopic Biopsy of Colonic Polyps

A Systematic Evaluation of Epithelial Misplacement With Review of Problematic Polyps From the Bowel Cancer Screening Program, United Kingdom

Nicole C. Panarelli, MD, Thusitha Somarathna, MB, BS, MD,† Wade S. Samowitz, MD,‡ Susan Kornacki, MD,§ Scott A. Sanders, MD, FRCPath,‖ Marco R. Novelli, MB, ChB, PhD, FRCPath,¶ Neil A. Shepherd, DM, FRCPath,† and Rhonda K. Yantiss, MD‖#*

Abstract: Endoscopic mucosal biopsy may misplace mucosal elements into the submucosa of colonic adenomas, mimicking invasive adenocarcinoma. Biopsy-related misplacement can be more challenging to recognize than typical misplaced epithelium (pseudoinvasion) in pedunculated polyps. We compared the features of 16 polyps with biopsy-related misplaced epithelium with those of 10 adenomas with pseudoinvasion and 10 adenomas with invasive adenocarcinoma and performed Ki67 and p53 immunostaining on all cases. Features of misplaced epithelium in polyps referred to the Bowel Cancer Screening Program Expert Board in the United Kingdom were also evaluated for the same morphologic features. Biopsy-related epithelial misplacement occurred in adenomas throughout the colon and often appeared infiltrative (69%), including epithelial cells singly dispersed within reactive fibroinflammatory stroma or granulation tissue (44%). Misplaced epithelium displayed only low-grade cytologic features and was associated with extruded mucin (75%), tattoo pigment (63%), and misplaced normal glands (38%); scant lamina propria and muscularis mucosae were often present (88% and 44%, respectively). Cases referred to the Bowel Cancer Screening Program Expert Board also contained infiltrative-appearing misplaced epithelium (91%) that was cytologically low grade (72%), contained nondysplastic glands (11%), and showed other signs of injury. In contrast, misplaced epithelium in pedunculated polyps always had a lobular contour with a rim of lamina propria, hemorrhage, and/or hemosiderin. Invasive carcinomas showed malignant cytology and desmoplasia; most (70%) lacked features of trauma. Ki67 and p53 staining was patchy and weak in the misplaced epithelium, whereas invasive carcinomas showed increased staining for one or both markers. Pathologists should be aware that endoscopically manipulated adenomas may contain misplaced epithelium that simulates malignancy.

Key Words: pseudoinvasion, misplaced epithelium, malignant polyp, immunohistochemistry

(*Am J Surg Pathol* 2016;40:1075–1083)

Detection of invasive adenocarcinoma in colorectal adenomas is clinically important, as some cases are at risk for local recurrence and/or regional lymph node metastases.[1–3] Occasional adenomas contain benign submucosal epithelium that simulates invasive adenocarcinoma.[4] Most of these are pedunculated polyps in the distal colon that are subjected to torsion and intraluminal trauma, causing dysplastic crypts and lamina propria to herniate through the muscularis mucosae. Such cases usually contain lobules of "misplaced" adenomatous epithelium and lamina propria in the submucosa in association with fibrosis, hemorrhage, hemosiderin deposits, and acellular pools of mucin.[5,6] We have noticed similar findings in endoscopically manipulated adenomas, particularly in previously sampled sessile adenomas of the proximal colon. In this situation, misplaced glands bear a striking resemblance to invasive adenocarcinoma because they are commonly disrupted and show irregularly dispersed tubules or single cells

From the *Department of Pathology, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx; §Department of Pathology, New York University Medical Center; #Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY; ‡Department of Pathology, University of Utah, Salt Lake City, UT; †Gloucestershire Cellular Pathology Laboratory, Cheltenham General Hospital, Cheltenham; ‖Department of Cellular Pathology, Warwick Hospital, Warwick; and ¶Department of Histopathology, University College Hospital, London, UK.

Supported by the translational research program of Weill Cornell Medicine.

Presented, in part, at the United States and Canadian Academy of Pathology Annual Meeting, March 21 to 27, 2015, Boston, MA.

Conflicts of Interest and Source of Funding: The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

Correspondence: Nicole C. Panarelli, MD, Department of Pathology, Albert Einstein College of Medicine, Bronx 10467, NY (e-mail: npanarel@montefiore.org).

associated with fibroinflammatory changes. In fact, the Bowel Cancer Screening Program (BCSP) Expert Board was established in the United Kingdom to address profound diagnostic challenges related, in part, to epithelial misplacement in colonic adenomas.

To our knowledge, the features of biopsy-related epithelial misplacement in adenomas have not been systematically evaluated in the literature. The aim of this study was to describe the spectrum of histologic features that can be seen in adenomas with epithelial misplacement due to endoscopic manipulation. We compared the clinical and morphologic features of endoscopically manipulated colonic adenomas with those of adenomas with misplaced epithelium (pseudoinvasion) due to torsion, as well as adenomas containing invasive adenocarcinoma (malignant polyps). All 3 types of polyps were evaluated for patterns of Ki67 and p53 immunoexpression in the submucosal epithelium to determine whether these markers facilitate distinction of biopsy-related changes from cancer. We also performed a review of the BCSP Expert Board database to characterize polyps referred for consensus and identify features that aid the distinction of benign submucosal epithelium from invasive carcinoma.

MATERIALS AND METHODS

Case and Control Selection

We evaluated hematoxylin and eosin–stained slides from 44 endoscopically or surgically resected adenomas, all of which were endoscopically sampled before complete excision. Sixteen (36%) of these polyps were included in the study because they showed dysplastic epithelium in the submucosa. We also identified 20 controls, including 10 pedunculated adenomas with epithelial misplacement and 10 malignant polyps containing invasive adenocarcinoma in the submucosa (pT1). Clinical information regarding patient demographics and endoscopic findings was obtained from the electronic medical record. This study was conducted following approval of the institutional review board of Weill Cornell Medicine.

Histologic Evaluation

Study cases and controls were entirely submitted for histologic evaluation and routinely processed (10% buffered formalin). Hematoxylin and eosin–stained sections were reviewed and the surface and submucosal epithelium separately evaluated. Dysplasia was classified as low or high grade. Crowded glands lined by cells with enlarged, hyperchromatic, and pseudostratified nuclei with inconspicuous nucleoli were considered to represent low-grade dysplasia; loss of cell polarity, cribriform or fused glands, round nuclei, nucleolar prominence, and open chromatin were considered to represent high-grade dysplasia. The submucosal epithelium was classified as circumscribed when comprising lobules of glands with a smooth external contour or as infiltrative when glands or single cells were irregularly dispersed. The presence, or absence, of nondysplastic glands, lamina propria, and/or muscularis mucosae in the submucosa was recorded. Cases were also evaluated for other alterations suggestive of previous trauma—namely, erosions or ulcers, fibroinflammatory or granulation tissue, extruded mucin in the submucosa, ruptured crypts with pericryptal inflammation, and hemorrhage and/or hemosiderin deposits. Invasive adenocarcinomas were graded and staged according to the *AJCC Cancer Staging Manual, Seventh Edition*.[7]

Immunohistochemical Studies

Immunoperoxidase studies were performed on 4-μm-thick paraffin-embedded tissue sections using standard techniques. Cases were stained with Ki67 (clone: MIB-1; Dako, Carpinteria, CA) at a 1:50 dilution and p53 (clone: 1801; Biogenix, Fremont, CA) at a 1:200 dilution. The Ki67 staining reaction in the submucosal epithelium was compared with that of the surface, and the percentage of positive nuclei in the most densely stained area of each compartment was recorded. Staining was considered to be similar in both areas if the difference in percentage of stained cells was $<20\%$ when comparing the surface with submucosal epithelium. Only strong nuclear staining for p53 was considered a positive result.

BCSP Expert Board Case Selection

At the time this study was conducted, the BCSP Expert Board (N.A.S., S.A.S., and M.R.N.) had reviewed over 200 polypectomy specimens referred from regional pathologists throughout the United Kingdom with a differential diagnosis of adenocarcinoma versus epithelial misplacement. All 3 board members reached a consensus diagnosis of misplaced epithelium in 64 cases. These polyps were deliberately selected for inclusion in the study because we felt a reasonably confident diagnosis of epithelial misplacement could be made, despite the challenging issues necessitating referral to the BCSP Expert Board. All 64 polyps were evaluated for the histologic features enumerated above.

RESULTS

Clinical Features of Study Cases

The clinical and endoscopic features of the 16 study cases and controls are summarized in Table 1. There were 12 men and 4 women in the study group with a mean age of 72 years (range, 55 to 87 y). Sixty-nine percent of these polyps were located proximal to the splenic flexure, and only 26% occurred in the sigmoid colon. All of the study polyps were interpreted to be adenomas on prior sampling, which was generally performed within 3 months before complete excision (mean: 49 d, median: 42, range: 1 to 97 d). These lesions were large (mean: 34 mm, range: 13 to 62 mm), and most (81%) had a sessile endoscopic appearance. Twelve (75%) polyps in the study group were present in colonic resection specimens, all of which were adequately staged with ≥ 12 negative lymph nodes. The other 4 cases consisted of endoscopically obtained polypectomy material.

TABLE 1. Clinical and Endoscopic Features of Study Cases and Controls

Features	n (%)		
	Adenomas With Biopsy-related Misplaced Epithelium (N = 16)	Adenomas With Misplaced Epithelium (Pseudoinvasion) (N = 10)	Adenomas With Invasive Adenocarcinoma (Malignant Polyp) (N = 10)
Mean age (y)	72	62	62
Male/female ratio	3/1	3/7	2/3
Polyp site			
Cecum	3 (19)	0	4 (40)
Ascending colon	5 (31)	0	0
Transverse colon	3 (19)	0	2 (20)
Descending colon	1 (6)	0	2 (20)
Sigmoid colon	2 (13)	9 (90)	1 (10)
Rectum	2 (13)	1 (10)	1 (10)
Endoscopic appearance			
Mean size (mm)	34	28	35
Sessile	13 (81)	0	5 (50)
Pedunculated	3 (19)	10 (100)	5 (50)
Definitive therapeutic intervention			
Endoscopic polypectomy	4 (25)	10 (100)	4 (40)
Surgical resection	12 (75)	0	6 (60)
Tumor stage			
Stage I	NA	NA	9 (90)
Stage II	NA	NA	0
Stage III	NA	NA	1 (10)
Stage IV	NA	NA	0

NA indicates not applicable.

There were 3 men and 7 women in the control group of patients with pedunculated adenomas with epithelial misplacement; these patients were older adults (mean age: 62 y, range: 47 to 89 y), comparable to the study group. All of the polyps occurred in the sigmoid colon and spanned at least 10 mm (mean: 28 mm, range: 15 to 50 mm, $P > 0.05$ compared with study cases). All of the control polyps were completely removed endoscopically.

Malignant polyps occurred in a similar age group; the mean patient age was 62 years (range: 44 to 85 y), and most patients were women (male/female: 2/3). Polyps were evenly distributed throughout the colon and were similar in size compared with those of the study group (mean: 35 mm, range: 15 to 65 mm). Five (50%) malignant polyps were sessile, and 5 were pedunculated. Six patients were treated with segmental resection following an initial diagnosis of adenocarcinoma. Segmental resection was prompted by inadequate margin clearance in 4 cases, high-grade cytologic features in 1 case, and the presence of multiple adenomas in another case. One of these tumors was associated with a single lymph node metastasis.

Pathologic Features of Study Cases and Controls

The pathologic features of study cases and controls are summarized in Table 2. Endoscopic manipulation produced 2 morphologic patterns of epithelial misplacement: circumscribed and ill-defined aggregates of epithelium in the submucosa. Circumscribed lobules were present in the submucosa of 5 (31%) cases. Four of these contained pools of mucin that harbored villi lined by dysplastic epithelium and supported by lamina propria (Fig. 1A). One of these polyps showed high-grade dysplasia in the surface epithelium adjacent to the biopsy site, but all showed low-grade dysplasia in the submucosal elements. Circumscribed lobules of adenomatous epithelium were accompanied by similarly misplaced nondysplastic colonic crypts and lamina propria in 2 cases. Other biopsy site changes included hemorrhage and hemosiderin deposits in 2 cases, granulation tissue in 1 case, and tattoo pigment in 3 cases.

Eleven (69%) study cases contained ill-defined clusters of glands in the submucosa that simulated invasive carcinoma (Fig. 1B). One of these showed high-grade dysplasia in the overlying polyp surface, although none showed high-grade cytologic features in the submucosal epithelium. Six polyps contained single cells or small (< 5 cells) clusters of epithelial cells dispersed in inflamed, fibrotic stroma (Fig. 1C). Two cases showed linear arrays and clusters of epithelial cells confined to fibrin and granulation tissue at the biopsy site (Fig. 1D). Infiltrative-appearing glands and single cells were associated with lamina propria in 9 (82%) of 11 polyps, although it was often scant and discontinuous around angulated glands (Figs. 1E, F). Single and clustered epithelial cells were associated with inflamed, ruptured crypts (55%) or mucin pools (73%) embedded in inflamed stroma (Figs. 2A–D). Misplaced, non-neoplastic epithelium was identified in 4 (36%) cases. Other biopsy site–related changes included erosions (45%), disrupted bundles of smooth muscle cells comingled with misplaced epithelium (64%), fibroinflammatory stroma (36%), granulation tissue (18%), hemorrhage and hemosiderin deposits (18%), and tattoo pigment (64%).

TABLE 2. Pathologic Features of Study Cases and Controls

	Study Cases (n [%])		Controls (n [%])	
Histologic Features	**Adenomas With Circumscribed Misplaced Epithelium (N = 5)**	**Adenomas With Infiltrative Misplaced Epithelium (N = 11)**	**Adenomas With Misplaced Epithelium (Pseudoinvasion) (N = 10)**	**Adenomas With Invasive Adenocarcinoma (Malignant Polyp) (N = 10)**
Adjacent surface epithelium				
Low-grade dysplasia	4 (80)	10 (91)	7 (70)	5 (50)
High-grade dysplasia	1 (20)	1 (9)	3 (30)	5 (50)
Submucosal epithelium				
Low-grade cytologic atypia	5 (100)	11 (100)	9 (90)	8 (80)
High-grade cytologic atypia	0	0	1 (10)	2 (20)
Single cells/clusters	0	6 (55)	0	2 (10)
Associated mucosal elements				
Lamina propria	5 (100)	9 (82)	10 (100)	0
Muscularis mucosae	0	7 (64)	8 (80)	0
Displaced normal mucosa	2 (40)	4 (36)	0	0
Other findings in the submucosa				
Extruded mucin	4 (80)	8 (73)	2 (20)	1 (10)
Granulation tissue	1 (20)	2 (18)	0	0
Fibroinflammatory tissue	0	4 (36)	1 (10)	1 (10)
Hemorrhage/hemosiderin	2 (40)	2 (18)	10 (100)	1 (10)
Erosions or ulcers	0	5 (45)	0	2 (20)
Inflamed, ruptured crypts	0	6 (55)	0	3 (30)
Tattoo	3 (60)	7 (64)	0	0

NA indicates not applicable.

The control group of pedunculated adenomas with misplaced epithelium showed features similar to those of endoscopically manipulated polyps with circumscribed lobules of submucosal epithelium. Three (30%) controls showed high-grade dysplasia in the surface epithelium, but only 1 contained high-grade dysplastic epithelium in the submucosa. Lobules of misplaced epithelium were surrounded by a smooth and continuous rim of lamina propria. Two polyps contained pools of extruded mucin, including 1 with dysplastic glands floating in mucin. Hemorrhage and hemosiderin deposits were present in all control adenomas with misplaced epithelium.

Eight (80%) adenocarcinomas were entirely low grade, and 2 (20%) contained rare singly infiltrating cells and buds of <5 epithelial cells. All adenocarcinomas contained angulated, irregularly arranged glands with desmoplasia, and 7 (70%) had an infiltrative border at the invasive front. Fifty percent of malignant polyps showed high-grade cytologic atypia in the surface epithelium, and 2 (20%) displayed surface erosions. Three (30%) contained ruptured crypts with associated inflammation. Extruded mucin and fibrosis were present in 1 case each.

Immunohistochemical Studies

Fourteen study polyps were evaluated with immunostains for Ki67 and p53; 2 cases were received in consultation, and additional material was not available. All of the study polyps showed a similar patchy distribution of Ki67 labeling in the misplaced epithelium compared with the surface adenoma, and none showed strong p53 staining in the submucosal epithelium; misplaced single cells were negative for Ki67 and p53 (Figs. 2E, F, 3A–F). These results were similar to those of control adenomas with epithelial misplacement, which showed similar (70%) or decreased (30%) Ki67 labeling in the misplaced epithelium compared with the surface adenoma. None of the adenoma controls showed strong p53 staining in the misplaced epithelium (Figs. 3G–I). In contrast, all invasive carcinomas showed increased staining for Ki67, p53, or both markers compared with adjacent adenomatous epithelium (Figs. 3J–L). Eighty percent of carcinomas showed more extensive Ki67 labeling in the invasive component than was present in the overlying adenoma, and 60% showed strong, diffuse p53 staining of invasive adenocarcinoma compared with dysplasia at the polyp surface.

Clinical Outcome

Follow-up information was available for 15 study patients with a mean interval of 9 months (range: 2 to 35 mo). Seven underwent a subsequent endoscopic examination that revealed additional adenomas in 4 patients, a hyperplastic polyp in 1 patient, and normal findings in 2 patients. The remaining 8 patients reported no gastrointestinal symptoms and had normal physical examinations. Follow-up information was available for 6 adenoma controls (mean: 13 mo, range: 3 to 32 mo). Four of these patients had another colonoscopic or sigmoidoscopic examination that revealed tubular adenomas (1 patient), diverticulosis (2 patients), and normal findings (1 patient). One patient experienced diarrhea that resolved with supportive care, and the last had a normal physical examination and was referred to her private gastroenterologist for follow-up colonoscopy. Eight control patients with malignant polyps received follow-up care at our institution (mean: 12 mo, range: 1 to 43 mo). One

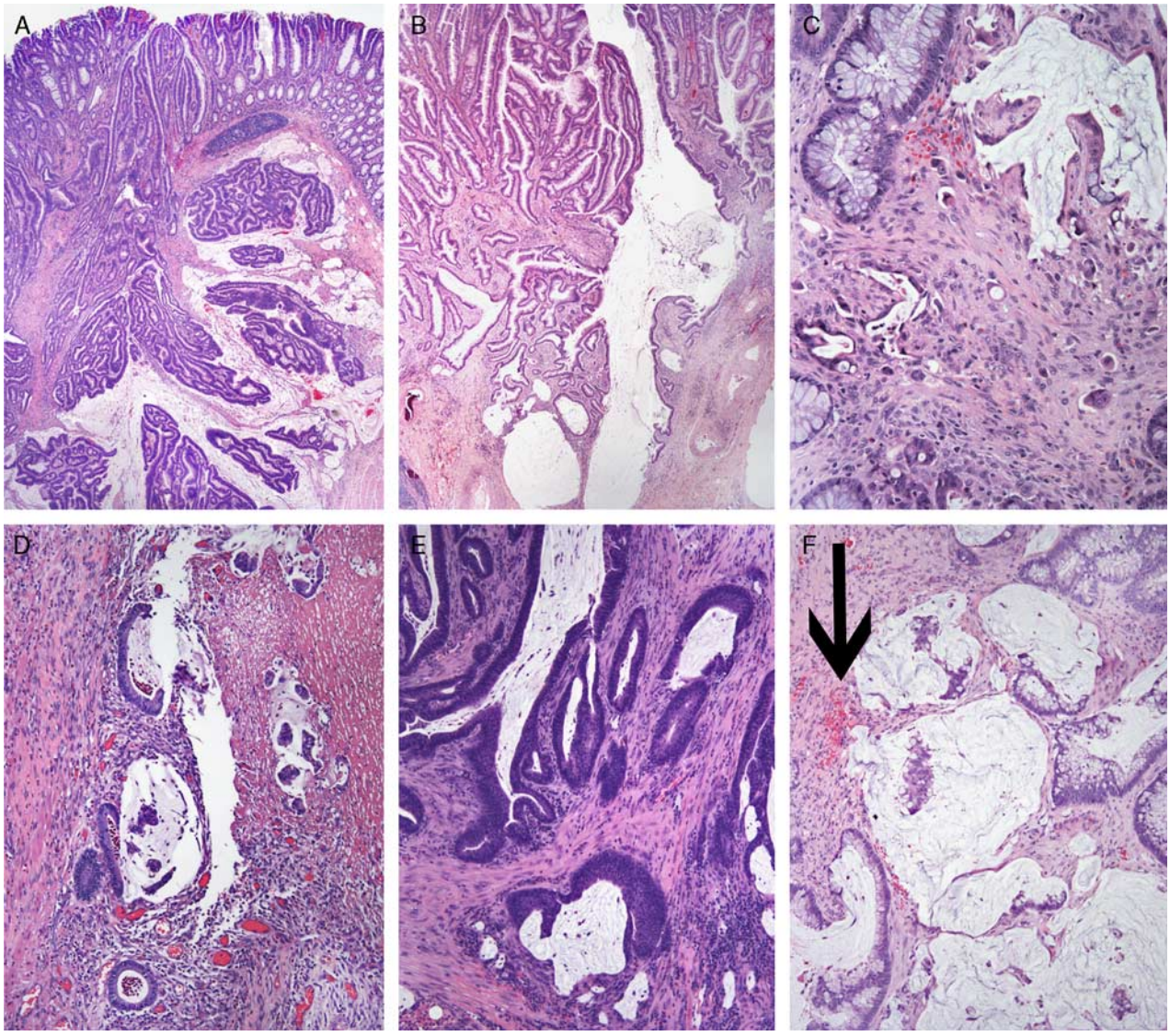

FIGURE 1. A previously sampled adenoma displays well-circumscribed lobules of misplaced mucosal elements in the submucosa. Pools of mucin contain low-grade neoplastic epithelium supported by lamina propria (A). Another case contains jagged, irregular glands in the submucosa. However, this focus is continuous with the surface of the polyp and is invested with lamina propria, supporting a benign diagnosis (B). The biopsy site of this adenoma contains irregularly shaped glands adjacent to benign, adenomatous epithelium (left). Single cells and dilated glands lined by attenuated epithelium are invested with inflamed lamina propria, rather than desmoplasia (C). A recently sampled adenoma contains linear arrays of epithelium embedded in fibrin and granulation tissue at the biopsy site (D). Crypts at the healing biopsy site are somewhat angulated and surrounded by a fibroinflammatory tissue reaction; discontinuous lamina propria is inconspicuous (E). Mucin pools are associated with lamina propria (arrow) and benign glands; they contain floating and adherent strips of adenomatous epithelium (F).

patient with a malignant polyp in the sigmoid colon underwent a colonic resection and chemotherapy for a metachronous ascending colonic adenocarcinoma. Three had follow-up colonoscopic examinations; 1 was found to have an adenoma, and 2 had no additional polyps. One patient was readmitted to the hospital for abdominal pain that resolved; 1 had a normal serum carcinoembryonic antigen level at 1 month; 1 had a follow-up CT scan that showed only diverticulosis and the last, most recent, patient recovered uneventfully from surgery.

BCSP Expert Board Cases

The 64 cases referred to the BCSP Expert Board included 46 (72%) adenomas with low-grade dysplasia in the surface epithelium and 18 (28%) with high-grade dysplasia on the polyp surface; only 9 (14%) cases showed high-grade dysplasia in the misplaced epithelium. Strips and lobules of misplaced epithelium were surrounded by mucin in 8 (13%) cases, one of which contained single misplaced glands in the submucosa; 3 others showed hemorrhage or hemosiderin deposits.

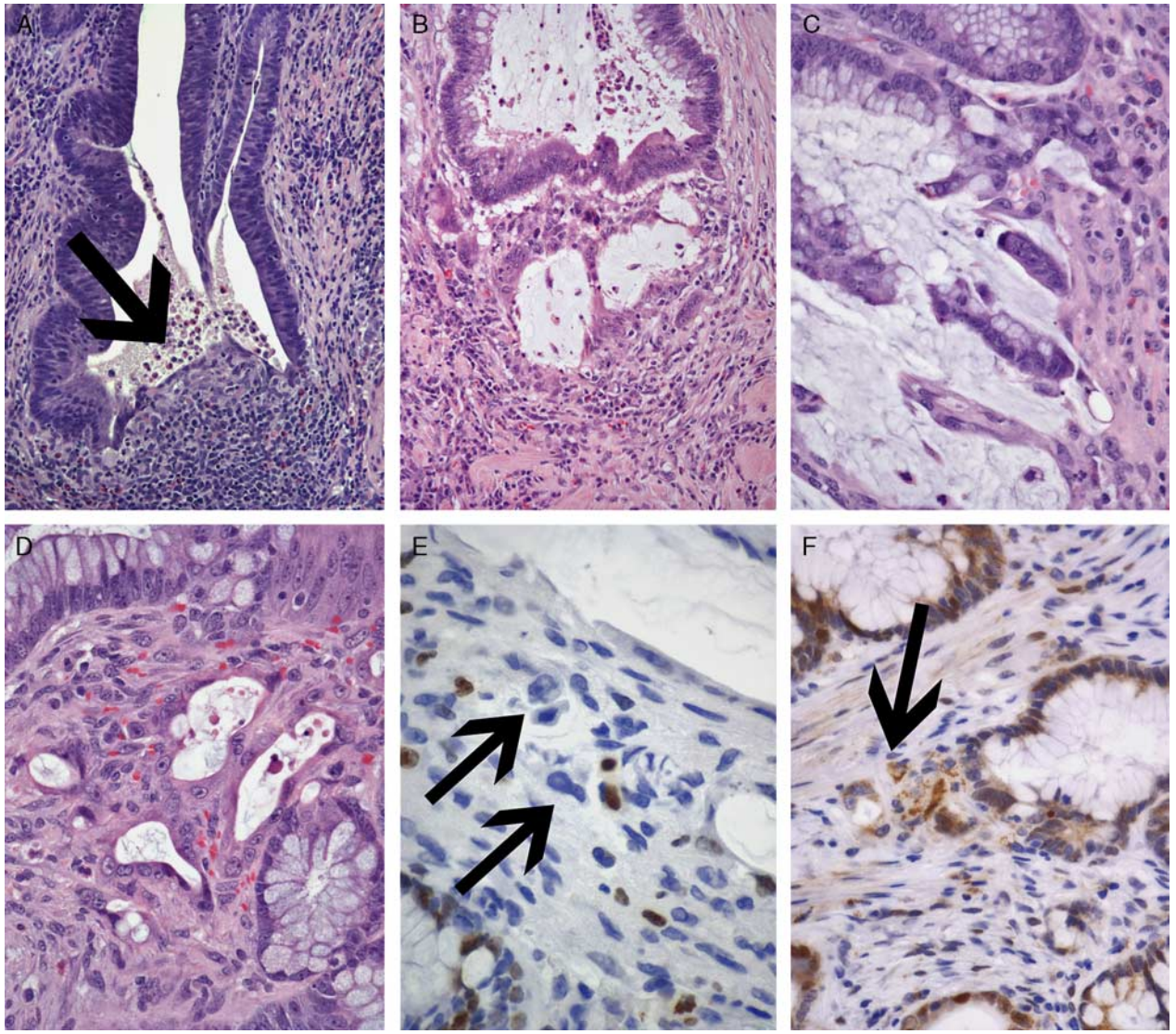

FIGURE 2. Granulomatous inflammation (arrow) is associated with a disrupted crypt in a previously sampled adenoma (A). Extruded mucin and cellular clusters are surrounded by inflamed lamina propria adjacent to an injured, benign crypt (B). Disrupted, benign crypts contain detached epithelial cells and are surrounded by inflamed lamina propria (C). Another case contains a cluster of atypical glands surrounded by lamina propria and benign epithelium (bottom right) (D). Dispersed single epithelial cells are negative for Ki67 (arrows), as are the attenuated cells lining irregularly shaped glands (E). An immunostain is negative for p53 in the same cell clusters (arrow), as well as intact adenomatous glands (F).

The remaining 56 (87%) cases contained irregularly dispersed dysplastic glands, single cells, and small clusters of epithelial cells in the submucosa. Lymphatic channels and/or small blood vessels contained misplaced epithelial cells and lamina propria in 3 (5%) cases. Seven cases (13%) displayed misplaced nondysplastic epithelium. Lamina propria and muscularis mucosae were associated with misplaced epithelium in 48 (86%) and 50 (89%) cases, respectively. Other trauma-related features included fibrosis (80%), granulation tissue (45%), acellular mucin pools (39%), fibroinflammatory reaction (36%), hemorrhage or hemosiderin deposits (34%), and tattoo pigment (5%).

DISCUSSION

The purpose of this study was to describe the spectrum of changes that can be seen in endoscopically manipulated adenomas. We found that biopsy-related epithelial misplacement into the submucosa is common, occurring in more than one-third of previously sampled adenomas. Most cases are large, sessile lesions of the proximal colon that are difficult to remove or are not amenable to resection by conventional endoscopic techniques. Endoscopic manipulation distorts and disrupts the epithelium, producing the appearance of infiltrative glands and single neoplastic cells in the submucosa. Features that aid distinction of biopsy-related epithelial

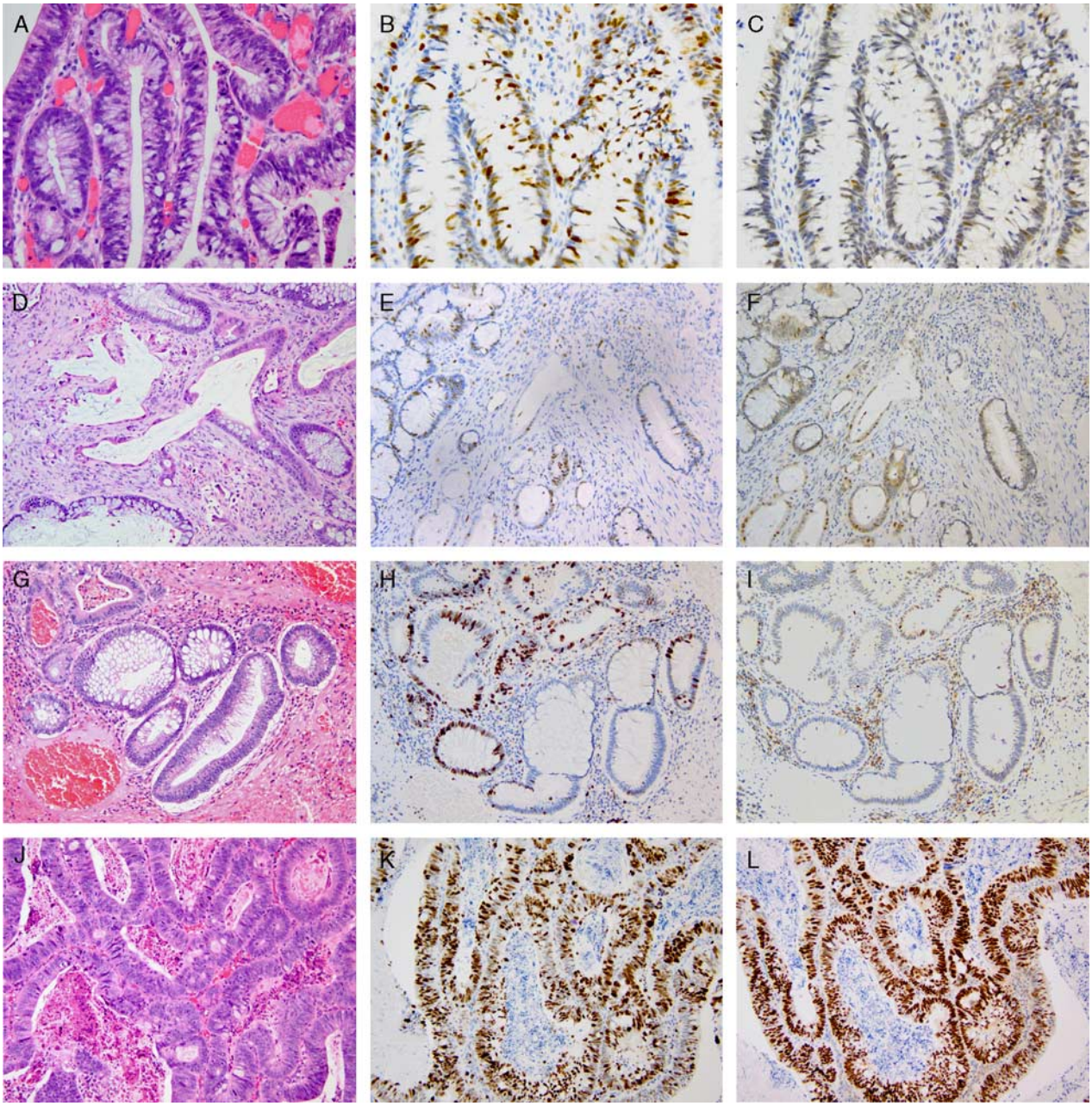

FIGURE 3. The surface of this adenoma shows low-grade dysplasia (A). Irregularly dispersed Ki67-positive nuclei are present throughout the epithelium (B). Rare nuclei show faint staining for p53 (C). Biopsy-related misplaced epithelium displays an infiltrative appearance, including disrupted glands with extruded mucin and single dispersed cells in the fibrotic stroma (D). The Ki67 immunostain reveals low proliferation (E), and p53 labels scattered nuclei (F), similar to that seen in the surface adenoma. A control adenoma with misplaced epithelium shows low-grade dysplasia associated with hemorrhage and hemosiderin (G). The Ki67 (H) and p53 (I) staining patterns are similar to those of biopsy-related misplaced epithelium. This case of adenocarcinoma contains fused glands and malignant cells with high nuclear-to-cytoplasmic ratio (J). The Ki67 (K) and p53 (L) immunostains show strong diffuse nuclear labeling for both markers.

misplacement from invasive carcinoma include the presence of lamina propria and muscularis mucosae around the submucosal epithelium, low-grade cytologic features, and concomitant nondysplastic epithelium in the submucosa. Biopsy site changes, such as extruded mucin, ruptured crypts with inflammation, granulation tissue, and fibrin, are often evident around the misplaced epithelium, as are hemosiderin deposits and extravasated red blood cells. Most examples of biopsy-related misplaced epithelium are mildly proliferative (ie, strong Ki67

labeling is patchy, or absent) and do not show strong p53 staining, in contrast to invasive carcinomas that usually show strong, diffuse staining for both markers.

Although poorly documented in the literature, diagnostic problems produced by endoscopic manipulation of adenomas are well recognized. Early data from the BCSP Expert Board showed disagreement with the original pathologic diagnosis in 24% of adenomas with misplaced epithelium.[8] The BCSP Expert Board attributed diagnostic difficulties to inflammatory changes around disrupted crypts that simulated tumor budding, obscured the lamina propria, and resembled desmoplasia.[9] Detailed assessment of 64 such cases referred to the BCSP Expert Board is provided herein: irregularly dispersed glands and/or single cells were present in 91% of cases, and fibrosis and inflammation were common (73% and 33%, respectively). Rare (5%) polyps subjected to endoscopic manipulation even contained misplaced epithelial cells (and lamina propria) within blood vessels and lymphatic channels. Similar to findings in the study cases, misplaced epithelium with low-grade dysplasia (72%) or no dysplasia (11%), extruded mucin (34%), and prolapsed elements of the muscularis mucosae (78%) provided helpful clues to the correct diagnosis. Most cases also showed other trauma-induced changes, including hemorrhage (34%), granulation tissue (39%), and ulcers (22%), to support the consensus diagnosis of benign epithelial misplacement.

The differential diagnosis of biopsy-related epithelial misplacement in colonic adenomas includes epithelial misplacement due to torsion and invasive adenocarcinoma. The former occurs almost exclusively in pedunculated adenomas of the distal colon.[5,6,10,11] Presumably, intermittent ischemic injury in combination with traumatic forces causes herniation of mucosal elements into the submucosa, where they appear as well-circumscribed lobules of dysplastic glands surrounded by a rim of lamina propria, hemorrhage or hemosiderin deposits, submucosal fibrosis, and pools of mucin. Thirty-one percent of endoscopically manipulated adenomas in this study displayed similar lobules of submucosal epithelium, often with mucin pools and lamina propria. Unlike pedunculated adenomas with torsion-related epithelial misplacement, however, most ($\sim$80%) endoscopically manipulated polyps were sessile and located proximal to the splenic flexure. Hemorrhage and/or hemosiderin deposits were not consistently present, although most (63%) contained tattoo pigment in close association with misplaced epithelium.

Sixty-nine percent of endoscopically manipulated polyps with misplaced epithelium closely mimicked invasive adenocarcinoma; they contained irregularly arranged neoplastic glands and single cells associated with a fibroblastic proliferation that simulated desmoplasia. Of note, all of the misplaced epithelium, including single cells and small cell clusters, showed low-grade cytologic features; dyscohesive cell groups were adjacent to partially denuded, or disrupted, benign crypts invested with lamina propria. Misplaced epithelium was associated with lamina propria in 88% of cases, and similarly misplaced normal (non-neoplastic) mucosal elements were present in 38% of cases. Other helpful indicators of recent trauma included pools of mucin (75%), fibrin, organizing granulation tissue at the biopsy site (44%), and splayed clusters of smooth muscle cells emanating from the muscularis mucosae (64%).

One may argue that some of our cases represent superficially invasive, well-differentiated adenocarcinomas. Gonzalez et al recently described 35 "adenoma-like" colonic adenocarcinomas that displayed low-grade cytology in biopsy samples.[12] However, the cytologic and architectural features of those cases are not at all similar to those of the current cases. All but 1 tumor, in that series, contained areas of traditional-appearing invasive adenocarcinoma with desmoplastic stroma, and, although 86% of cases were sampled before resection, the authors did not describe the biopsy site changes that we observed, nor are such changes depicted in their images. Moreover, 86% of tumors in that series proved to be invasive beyond the submucosa; 20% and 15% were associated with lymph node and distant metastases, respectively. Although one cannot definitively prove the benign nature of submucosal epithelium in our cases, circumstantial evidence supports a benign diagnosis. Clustered and single cells were generally associated with disrupted, benign-appearing crypts, inflammatory changes, and altered lamina propria. Confinement of atypical cells to granulation tissue and fibrin of the biopsy site also argues in favor of a benign interpretation. None of the prior biopsy samples from the cases in this series showed features worrisome for invasive adenocarcinoma, nor did any patients have clear-cut evidence of malignancy (eg, vascular invasion, lymph node metastases) in subsequent resection specimens or clinical follow-up.

Immunohistochemical stains may aid distinction between invasive carcinoma and misplaced epithelium. Yantiss et al evaluated the utility of p53 in distinguishing between adenomas with misplaced epithelium and malignant polyps. They found that adenomas with misplaced epithelium showed a similar weak, patchy pattern of p53 staining in both submucosal epithelium and the surface adenoma, whereas 61% of invasive carcinomas showed strong, diffuse staining for this marker.[5] Similar findings have been reported when biopsy-related changes simulate malignancy in other organ systems. For example, van Deurzen et al[13] observed weaker p53 immunostaining in isolated tumor cells compared with micrometastases and macrometastases in breast sentinel nodes, supporting the notion that isolated tumor cells represent benign misplaced epithelium. The results of the current study are similar; all examples of misplaced epithelium lacked strong p53 staining and showed limited Ki67 immunolabeling similar to that present in the adjacent surface adenoma, whereas most invasive carcinomas showed strong, diffuse staining for both markers. We conclude that the combination of Ki67 and p53 immunostains may be used to confirm a benign diagnosis in previously sampled polyps. However, we suspect they are of limited value when high-grade dysplastic epithelium is introduced into the submucosa by endoscopic manipulation, as this benign epithelium could show an immunoexpression pattern identical to that of invasive adenocarcinoma.

In summary, endoscopic biopsy of colonic adenomas frequently introduces dysplastic epithelium into the submucosa. This misplaced epithelium may be confused with invasive adenocarcinoma because it often has an infiltrative appearance, including single cells, resulting from gland disruption. Helpful clues to a benign diagnosis include close proximity of worrisome epithelium to ruptured, inflamed crypts, discontinuous foci of lamina propria and muscularis mucosae around the epithelium, extruded pools of mucin, and tattoo pigment. Confinement of atypical epithelium to fibrin and granulation tissue is not a characteristic feature of cancer and should prompt alternative explanations for the findings. Immunohistochemical stains for Ki67 and p53 may be helpful in select cases, as benign submucosal epithelium shows minimal staining, whereas most cancers show strong, extensive staining for one, or both, markers. Pathologists should be aware of the spectrum of changes that can be seen in previously sampled adenomas, in order to avoid a misdiagnosis of invasive adenocarcinoma.

REFERENCES

1. Haggitt RC. Management of the patient with carcinoma in an adenoma. *Prog Clin Biol Res*. 1988;279:89–99.
2. Haggitt RC, Glotzbach RE, Soffer EE, et al. Prognostic factors in colorectal carcinomas arising in adenomas: implications for lesions removed by endoscopic polypectomy. *Gastroenterology*. 1985;89:328–336.
3. Choi DH, Sohn DK, Chang HJ, et al. Indications for subsequent surgery after endoscopic resection of submucosally invasive colorectal carcinomas: a prospective cohort study. *Dis Colon Rectum*. 2009;52:438–445.
4. Muto T, Bussey HJ, Morson BC. Pseudo-carcinomatous invasion in adenomatous polyps of the colon and rectum. *J Clin Pathol*. 1973;26:25–31.
5. Yantiss RK, Bosenberg MW, Antonioli DA, et al. Utility of MMP-1, p53, E-cadherin, and collagen IV immunohistochemical stains in the differential diagnosis of adenomas with misplaced epithelium versus adenomas with invasive adenocarcinoma. *Am J Surg Pathol*. 2002;26:206–215.
6. Pascal RR, Hertzler G, Hunter S, et al. Pseudoinvasion with high-grade dysplasia in a colonic adenoma. Distinction from adenocarcinoma. *Am J Surg Pathol*. 1990;14:694–697.
7. Edge SB, Byrd DR, Compton CC, et al. *AJCC Cancer Staging Manual*, 7th ed. New York: Springer; 2010.
8. Shepherd NA, Griggs RK. Bowel cancer screening-generated diagnostic conundrum of the century: pseudoinvasion in sigmoid colonic polyps. *Mod Pathol*. 2015;28(suppl 1):S88–S94.
9. Loughrey MB, Shepherd NA. The pathology of bowel cancer screening. *Histopathology*. 2015;66:66–77.
10. Tanizawa T, Seki T, Nakano M, et al. Pseudoinvasion of the colorectal polypoid tumors: serial section study of problematic cases. *Pathol Int*. 2003;53:584–590.
11. Byun TJ, Han DS, Ahn SB, et al. Pseudoinvasion in an adenomatous polyp of the colon mimicking invasive colon cancer. *Gut Liver*. 2009;3:130–133.
12. Gonzalez RS, Cates JM, Washington MK, et al. Adenoma-like adenocarcinoma: a subtype of colorectal carcinoma with good prognosis, deceptive appearance on biopsy and frequent KRAS mutation. *Histopathology*. 2016;68:183–190.
13. van Deurzen CH, de Bruin PC, Koelemij R, et al. Isolated tumor cells in breast cancer sentinel lymph nodes: displacement or metastases? An immunohistochemical study. *Hum Pathol*. 2009;40:778–782.