Looking Through a Keyhole
Serrated Neoplasia in the Vermiform Appendix

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Appendectomy is one of the most common surgical procedures in the United States, usually performed on an emergency basis for signs and symptoms of acute appendicitis or incidentally during surgery for other conditions, with a minor subset of patients having a mass lesion of the appendix. Nevertheless, because the appendix is currently inaccessible by endoscopy, we have minimal information regarding the biologic potential and evolution of mucosal alterations in the appendix, except by inference. Other lumina in the gastrointestinal tract can be scrutinized with increasing sensitivity using white-light endoscopy or advanced endoscopic methods, including magnification endoscopy, chromoendoscopy, and other techniques, allowing lesions of interest to be biopsied and followed up. In contrast, the cellular events in the appendix of a given patient remain invisible until there is trouble. As a result, our knowledge of the biologic potential of epithelial alterations in this organ is akin to peering through a keyhole. Advances in knowledge have been made by painstaking reviews of series of patients with careful clinical follow-up in an attempt to develop a longitudinal understanding that would permit us to make sense of the changes we see at the microscope.

Contributing to the challenges is the relatively limited sampling that is standard in most institutions. The majority of published guidelines for histologic sampling recommend including a longitudinal section or cross-section of the appendiceal tip and 2 additional cross-sections, ideally of the middle portion and from the proximal margin of resection. Few institutions routinely sample the entire resected appendix, except when neoplasms are detected on examination of initial sections.

Located shortly beyond the ileocecal valve with its orifice opening into the cecum, the vermiform appendix is lined by mucosa histologically similar to colonic mucosa, lacking the villous architecture of the small intestine. Therefore, pathologists have traditionally chosen to apply terms used in pathologic diagnosis of colon to similar lesions found in the appendix. Nevertheless, the appendix is not the colon. Compared with the colon, the appendix has considerably more mucosal-associated lymphoid tissue, along with specialized follicle-associated epithelial units, including M cells, to present luminal antigens to the immune system. The extensive follicular architecture distorts the appendiceal crypts in many areas, leading to variable crypt length, distribution, and architecture, including focal interdigitation with lymphoid tissue or areas without crypts. Appendiceal crypts also contain scattered Paneth cells and numerous endocrine cells.1 Although the amount of lymphoid tissue varies with the age of the host, it has a role in host immunity and host defense from infections. Most recently, the concept that the appendix is a vestigial organ has lost ground as attention had turned to the role of the microbiome. It is now known that there are many previously undiscovered species of bacteria residing in the gastrointestinal tract, and the appendix may be an important reservoir of microfilms of gut flora from which intestinal recolonization can occur following infection or other stresses.2 Finally, in addition to the specialized features of the appendiceal mucosa, the organization and distribution of nerves in the lamina propria and the muscular layers of the appendix differ from those of the colon.3

Some pathologic conditions are seen only in the appendix. Other lesions in the appendix may be histologically similar to colonic lesions but have different biologic behavior. Appendiceal mucinous cystadenoma (MCA), low-grade mucinous neoplasms with associated pseudomyxoma peritonei, and
some types of endocrine neoplasia have unique features not seen in other intestinal sites. Consequently, investigators of appendiceal pathology have focused on characterizing and delineating the histologic and biologic features of appendiceal neoplasms and how they differ from similar colonic neoplasms.

The interest in serrated lesions of the colon and appendix is long-standing. Lane et al. studied 2,136 small (<3 mm) colonic polyps and recognized the different histologic features of hyperplastic and adenomatous polyps. Hyperplastic polyps (HPs) had “orderly development of goblet cells” with “mucin secretion...most abundant in the superficial portion of the gland.” This contrasted with the adenomatous epithelium, in architectural organization and in the patterns of epithelial renewal, with mitotic activity located in the basal crypts in HPs, but with mitotic figures commonly noted in the surface and upper portions of adenomatous glands. These authors recognized that adenomas had malignant potential, in contrast with the benign nature of small HPs. Nevertheless, the discrete separation and categorization of HPs from adenomatous polyps were challenged by cases that displayed mixed phenotypes.

Goldman et al. observed foci of “hyperplastic-type glands” within 10 of 62 “villous adenomas,” raising the possibility that some HPs might lead to neoplastic growth. The photomicrographs provided for the villose adenomas have serrated luminal profiles, stratified nuclei, basal crypt serrations, altered basal crypt orientation, and other features now associated with sessile serrated adenomas (SSAs). One lesion has a region that resembles SSA and a region that had a “villose neoplastic portion” with cytologically neoplastic nuclei characterized by stratification and hyperchromasia. Among their conclusions was the statement that “evidence is provided for a possible neoplastic transformation of some HPs into villous adenomas.”

Around the same time, MacGillivray first described serrated lesions in the appendix, using the descriptor “mucosal metaplasia.” MacGillivray noted that “Variation in height of the gland.” This contrasted with the adenomatous epithelium, in architectural organization and in the patterns of epithelial renewal, with mitotic activity located in the basal crypts in HPs, but with mitotic figures commonly noted in the surface and upper portions of adenomatous glands. These authors recognized that adenomas had malignant potential, in contrast with the benign nature of small HPs. Nevertheless, the discrete separation and categorization of HPs from adenomatous polyps were challenged by cases that displayed mixed phenotypes.

More recently, Younes and colleagues studied the association between mucosal hyperplasia of the appendix and adenocarcinoma of the colon, reviewing appendiceal mucosa from 122 ileoectomy specimens and from 273 consecutive appendectomy specimens. As in the aforementioned studies, the photomicrographs of mucosal hyperplasia met criteria for SSAs. Among the ileoectomy specimens, 23 (18.9%) had mucosal hyperplasia of the appendix, and 18 of these (78%) were associated with colocolonic carcinoma, predominantly right-sided. Based on this association, the authors suggest that the presence of sessile serrated adenomatous lesions in an appendectomy specimen should prompt examination of the colon to exclude a concomitant colorectal carcinoma.

Thus, the stage was set for scrutiny of serrated lesions, with evidence from the colon and the appendix of possible association of some variant of mucosal hyperplasia with risk for adenocarcinoma. There has been a subsequent explosion of interest in serrated colonic polyps during the past 2 decades, fueled by 4 major developments. In 1990, Longacre and Fenoglio-Preiser described a specific type of colonic adenomas with serrated and hyperplastic features, so-called...
serrated adenomas, now commonly termed traditional serrated adenomas. In a parallel development, molecular analysis of colonic neoplasms associated with Lynch syndrome led to the discovery of the DNA mismatch repair genes and proteins.12,13 Patients with microsatellite unstable colon cancers (MSI-high) were observed to have increased numbers of hyperplastic or serrated colonic polyps. And serrated lesions were observed adjacent to some colonic adenocarcinomas. In the context of these discoveries, Torkulovic et al7 undertook a careful morphologic evaluation and statistical analysis of a large group of polyps previously termed “hyperplastic” and derived a set of morphologic features associated with abnormal proliferation and altered expression of mismatch repair proteins MLH-1 and MSH-2. Further studies of SSAs in the colon have identified BRAF mutations and DNA hypermethylation in CPG islands in these polyps and the association with microsatellite instable colorectal carcinomas. SSAs, unlike the traditional serrated adenoma, are not cytologically dysplastic, but rather have architectural features of altered crypt polarity and proliferation, leading to the appellation sessile serrated adenoma. Goldstein et al14 performed multivariate analysis of histologic features of serrated polyps associated with subsequent development of colonic adenocarcinoma near the site of the polyp, and the morphologic characteristics matched those defined by Torkulovic et al7 in SSAs. In addition, all cases in the series by Goldstein et al14 were MSI-high, and there was loss of nuclear expression of the mismatch repair protein MLH-1.

A number of investigators have now applied the aforementioned refined morphologic classification schemes for serrated mucosal lesions to the appendix to determine if there are lesions analogous to colonic SSAs and traditional serrated adenomas, with differing biologic potential from conventional adenomas. Rubio15 reviewed 38 noncarcinoid polyps or tumors of the appendix and identified 4 HPs, 10 serrated adenomas, 6 villous adenomas, and 8 mucinous adenocarcinomas. Among the serrated adenoma group, 4 were associated with invasive adenocarcinoma. Renshaw et al16 investigated the incidence of SSA in patients with acute appendicitis, comparing 100 consecutive appendices that were entirely submitted for histologic examination with 100 routinely submitted appendices (partially sampled). Among appendices entirely sampled, 11 cases of SSA were identified, compared with 1 case in the routinely sampled group. The SSAs were small and present in 3 or fewer cross-sections, and the incidence was significantly higher \( (P = .001) \) in patients with acute appendicitis who were older than 30 years.16 This finding is similar to the age range described for mucosal hyperplasia in the study by Higa et al.9

The link between colonic serrated polyps and MSI-high colorectal carcinomas has piqued interest in the biology of appendiceal serrated polyps. In this month’s issue of the Journal, Bellizzi et al17 report their comparative study of 53 serrated lesions of the vermiform appendix, which they classified into categories that include HPs, SSA, mixed serrated and adenomatous lesion (MSAL), conventional adenoma, and MCA. Their study, incorporating immunohistochemical and morphologic studies, adds important new information to the work of other pathologists regarding the cellular changes and neoplasms of the appendix. Immunohistochemical markers applied included cytokeratin 20, the proliferation marker Ki-67, MUC6, and β-catenin. Recognizing the challenges of histologic separation of HPs from SSAs, Bellizzi et al17 considered HPs “roughly equivalent to focal mucosal hyperplasia (metaplasia) and sessile serrated adenoma to be roughly equivalent to diffuse mucosal hyperplasia (metaplasia),” but SSAs also had at least 20% of crypts with basal crypt dilatation or branching, crypts with transverse orientation with respect to muscularis mucosae, serratations extending to the crypt base, and the presence of diffuse mucous cells at the base, features that have been described as characteristic of SSAs in the colon.7 HPs tended to be smaller than SSAs, the latter often circumferential. Bellizzi et al17 identified a number of lesions with varying degrees of architectural serration and cytologic dysplasia; they appropriately used the category of MSAL to study them, acknowledging the difficulty of separating some lesions into pure or distinct diagnostic categories.

Pai and Longacre18 noted the challenges of distinguishing mucinous cystic adenomas, in some circumstances, from serrated adenomas or SSAs because lesions of both types may have villiform architecture and a serrated surface. One of the strengths of the article by Bellizzi et al17 is the inclusion of a group of MCAs. The study found certain clear differences between MCA and serrated lesions. The mean age of patients with MCA was 48 years, whereas the mean age of patients with other polyp types was more than 62 years, a statistically significant difference. MCA has a higher incidence in women, whereas the serrated lesions appear roughly similar in sex distribution. MUC6 staining was expressed differently in MCA compared with serrated lesions, including HPs, SSAs, and MSALs. None of the 14 MCAs expressed MUC6. In contrast, MUC6 was expressed in 100% of the SSAs \( (n = 14) \), in 17% of HPs \( (1/6) \), and in 50% of MSALs \( (8/16) \). The reported expression of MUC6 in appendiceal SSA is similar to what has been reported in colonic SSA.19 These findings add support to separation of MCA from serrated lesions. MCAs are associated with mucinous neoplasms, including risk for pseudomyxoma peritonei, whereas SSA and MSAL may be found with other types of advanced neoplasia.

With respect to molecular characterization of serrated polyps of the appendix, the work is just beginning, and the numbers of cases studied are small. Bellizzi et al17 did not observe abnormal nuclear localization of β-catenin in any of the cases of HP, SSA, or MSAL, an observation confirmed in a separate study of appendiceal serrated lesions by Yantiss.
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and colleagues. Yantiss et al compared a group of 56 serrated appendiceal polyps with 17 MCAs and 4 adenocarcinomas with adjacent serrated appendiceal polyps for MLH-1, MSH-2, MGMT, β-catenin, p53, and Ki-67 expression, BRAF and KRAS mutations, and microsatellite instability. They found that loss of MLH-1 was frequent in serrated polyps but was unassociated with MSI-H in any of the cases. BRAF mutations were found in 29% of cases, whereas 34% had KRAS mutations. Serrated lesions with dysplasia were less frequently associated with BRAF mutations. Carcinomas adjacent to serrated polyps comprised only 4 cases, and there was no clear pattern of abnormalities in MLH-1 or MGMT expression, MSI, or BRAF mutations. The work by Yantiss and colleagues implies that the molecular characteristics of appendiceal serrated lesions may differ from those in the colon, with no evidence for a clear BRAF mutation or MSI-high pathway; however, the number of cases studied was small. Considering that hereditary nonpolyposis colorectal carcinoma is seen in only about 3% of colorectal carcinoma cases, many appendiceal neoplasms are needed to define genetic and molecular events with clarity.

Further elucidation of the biologic potential and molecular phenotype of serrated lesions in the appendix awaits additional work, careful gross examination and histologic sampling, more cases, and long-term follow-up. In the meantime, we will continue to look into a keyhole, assembling clues and insights.

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References