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CONTEMPORARY ISSUES IN LOWER GASTROINTESTINAL PATHOLOGY:
WHAT YOU NEED TO KNOW TO SURVIVE

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INTERPRETATION OF COLONIC BIOPSY SPECIMENS IN PATIENTS SUSPECTED OF HAVING INFLAMMATORY BOWEL DISEASE

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Introduction:

With increased availability of total colonoscopy and flexible sigmoidoscopy, pathologists can expect an ever-increasing number of colorectal biopsy specimens. The pathologist plays a critical role in the diagnosis and management of patients with colitis and suspected colitis (1). The numerous uses of mucosal biopsy in the evaluation of these conditions are summarized in Table 1.

<table>
<thead>
<tr>
<th>TABLE 1 - USES OF COLONIC MUCOSAL BIOPSY IN THE EVALUATION OF COLITIS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Documentation of colitis.</td>
</tr>
<tr>
<td>2. Identification of specific forms of colitis (e.g., collagenous colitis, ischemic colitis).</td>
</tr>
<tr>
<td>3. Documentation of the severity, extent, and nature (focal vs. diffuse) of inflammation.</td>
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<td>4. To follow the clinical course of disease.</td>
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<td>5. Surveillance for dysplasia and carcinoma.</td>
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<td>6. Detection of conditions that mimic inflammatory bowel disease (e.g., solitary rectal ulcer syndrome).</td>
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</tbody>
</table>

*From reference 1.

When evaluating a biopsy specimen, it is useful to scan the tissue at low magnification and ask several questions. Are abnormalities present? If present, are the changes diffuse or focal? Is the luminal border of the specimen straight or irregular? Is intraepithelial mucin (the goblet cell population) preserved or depleted? If intracellular mucin is depleted, is this change focal or diffuse? Are inflammatory cells increased in the lamina propria? Is this increase diffuse or focal? What kind of inflammatory cells are they? Is the lamina propria obliterated by fibrous or fibromuscular tissue? Are crypt abscesses present? Are colonic tubules straight? Do the colonic tubules reach the muscularis mucosae or are they short, branched, or budding?

With careful consideration of the features outlined above, one can often recognize a pattern of abnormality that when coupled with clinical and endoscopic information can lead to a fairly specific diagnosis.

Normal Colonic Histology (2)

The normal colonic luminal surface is straight. Colonic tubules are tightly packed, parallel, and non-branching, and all closely approximate the muscularis mucosae. The appearance is similar to test tubes in a rack. Goblet cells are numerous. The lamina propria contains a modest amount of mixed inflammatory cells including plasma cells, lymphocytes, eosinophils and macrophages. An occasional intraepithelial lymphocyte can be seen. The muscularis mucosae is thin and regular. The submucosa is generally devoid
of inflammation. Scattered intramucosal lymphoid follicles are normally encountered, especially in younger individuals. In areas of lymphoid follicles, the architecture of the mucosa may be mildly distorted, the muscularis mucosae may be incomplete, and some of the lymphoid follicles may spill over into the superficial portion of the submucosa. Overlying the lymphoid aggregates are flattened surface epithelial cells termed "M" cells. In this M cell region the epithelium normally contains more mononuclear inflammatory cells (2,3), and the amount of intraepithelial mucin is decreased. Paneth cells can be seen in the base of colonic crypts but are considered a normal finding only in the cecum and proximal ascending colon (2,3).

Changes associated with bowel preparation or with the trauma of biopsy itself can be expected. Bowel preparation decreases the amount of intracellular mucin, causes a mild increase in the number of mitotic figures, causes surface degeneration in the form of apoptosis and can even be associated with rare neutrophils in surface epithelium and crypts (2-8). Edema and recent hemorrhage into tissue not associated with other degenerative or inflammatory changes are best attributed to biopsy trauma.

Muciphages (macrophages containing PAS-positive material) are often present within the lamina propria of the large intestine, especially in the lower rectum. Although some observers do not consider their presence abnormal since they do not correlate with any inflammatory or infiltrative disorder (9,9a), others think they represent a nonspecific response to mucosal injury (9a,10). Muciphages have been confused with Whipple's disease and with Mycobacterium Avium-Intracellulare Complex infection. The macrophages in Whipple's disease are mucicarmine-negative, and acid fast stains will be positive in mycobacterial infection.

**Chronic Colitis**

Quiescent ulcerative colitis best typifies the chronic colitis pattern of injury. The predominant features are mucosal atrophy and mucosal architectural distortion (11-14). The luminal border is irregular, the number of crypts decreased; in addition, the remaining crypts appear short (i.e., they do not touch the muscularis mucosae), lose their parallel arrangement, and become branched and budded. The goblet cell population is usually preserved. Chronic inflammatory cells are only mildly increased in the lamina propria. Paneth cells may be present. The muscularis mucosae is usually markedly hypertrophied. The above changes, although consistent with a diagnosis of chronic ulcerative colitis, must be interpreted in light of the clinical, radiologic and endoscopic findings because identical changes can be seen in focal, healed, or healing areas of other chronic colitides such as Crohn's disease, ischemia (including chronic irradiation injury), tuberculosis, and schistosomiasis.

Care must be taken when interpreting biopsy specimens obtained from the normal mucosa adjacent to lymphoid follicles, from normal mucosa containing the innominate groove, and from the lower portion of the rectum near the transition zone. These areas may normally show some loss of crypt parallelism and should not be misinterpreted as chronic colitis (8). Conversely, histologically normal biopsy specimens must not be reported as showing "chronic nonspecific inflammation consistent with chronic ulcerative colitis." The guidelines I favor are these; unless one or more of the features discussed in the preceding paragraph are also present, it is a good rule not to diagnose inflammatory bowel disease based only on an evaluation of inflammatory cells in the lamina propria (1).
Active Colitis

The term "active" colitis describes inflammatory conditions in which neutrophils are present in the lamina propria, within epithelial cells (cryptitis) or within crypt lumens (crypt abscesses). Included under this heading are ulcerative colitis in an active phase, most examples of Crohn's colitis and, infectious colitis/acute self-limited colitis (15,16).

Diffuse Active Colitis

Ulcerative colitis in an active phase represents the prototype diffuse active colitis. Biopsy specimens usually demonstrate diffuse abnormalities, meaning that changes are of approximately the same intensity in all areas of the tissue from a particular region of the colon. The luminal border of the mucosa is irregular (12-18). Increased numbers of chronic inflammatory cells are present in the lamina propria and may occasionally spill over into the superficial portion of the submucosa. Intracellular mucin in goblet cells is diffusely depleted (19). Cryptitis and crypt abscess formation are often prominent (18). It is surprising that even in ulcerative colitis of extremely short overt clinical duration, some atrophy, branching, and budding of crypts are already apparent in many specimens (8,13-18). This crypt distortion coupled with basal plasmacytosis (increased numbers of plasma cells in the lower fifth of the mucosa), has been proposed as the most useful histological criteria to differentiate ulcerative colitis from infectious colitis/acute self-limited colitis (12,15-17).

Remember, the most a pathologist can conclude from a biopsy specimen showing this pattern of injury is that changes are consistent with ulcerative colitis in an active phase. The diffuse active colitis pattern can also be seen in some examples of Crohn's colitis (13,17), and in some cases of documented infectious colitis (20). The diffuse active colitis can also be seen in a newly recognized form of colitis associated with diverticular disease (21); however, this entity can be distinguished from ulcerative colitis by its rectal sparing and its presence only in areas of diverticula.

Focal Active Colitis

The focal active colitis pattern of injury refers to the patchy distribution of architectural change and/or acute inflammation in a mucosal biopsy specimen. Chronic colitis showing diffuse chronic changes as described above, coupled with patchy acute inflammation, is not considered focal active colitis but is classified as chronic ulcerative colitis showing mild activity. The focal active colitis pattern consists of limited areas of increased inflammatory cells sometimes coupled with focal minimal architectural distortion; characteristically, some areas of the biopsy specimen maintain an essentially normal appearance. Focal active colitis is usually not seen with ulcerative colitis and when present suggests Crohn's colitis (8,22,23), infectious colitis, or acute self-limited colitis (see below) (8,12,13-16,19,23,24). Remember, however, that focal active colitis can be seen in resolving ulcerative colitis under active medical therapy (1,19,25) and that areas of previously involved colon/rectum in ulcerative colitis can return to an almost normal histologic appearance with therapy.

Differential Diagnosis

The major differential diagnostic features in biopsy specimens containing the diffuse and focal active colitis patterns are summarized in Table 2. Granulomas typically found in Crohn's disease should be sought in all biopsy specimens, but especially those showing the focal active pattern. Some authors advocate serial sectioning for their detection (26). In my experience, granulomas are rarely missed; however, germinal centers, tangential cuts of blood vessels, tangential cuts of the pericryptal fibroblastic sheath, and inflammatory reactions to extravasated mucin (mucin granulomas) are often misinterpreted as...
the granulomas of Crohn's disease (1). In the absence of true granulomas, biopsy specimens from patients with Crohn's disease often show the focal active colitis pattern without neutrophils in the lamina propria. However, some examples of Crohn's colitis may be indistinguishable from resolving mucosal ulcerative colitis in biopsy specimens (12,17).

A fibrinopurulent exudate in the specimen overlying but separate from the mucosa is always abnormal. The clinician must be informed that ulceration is likely to be present in a more proximal location in the bowel. As a practical note, all inflammatory exudates should be examined under high magnification for trophozoites of Entamoeba histolytica, because this is their preferred hiding place and they are easy to overlook.

The definitive classification of colonic inflammation depends upon clinical pathologic correlation. In my opinion, the task of the pathologist is to convey the histologic pattern of injury to the clinician who then collates that information with the clinical history and data obtained from endoscopic, radiologic and laboratory examination. Through consideration of this information an accurate diagnosis can often be rendered.

**TABLE 2 - DIFFERENTIAL FEATURES IN BIOPSY SPECIMENS DEMONSTRATING THE ACTIVE COLITIS PATTERN**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Ulcerative Colitis</th>
<th>Resolving Ulcerative Colitis</th>
<th>Crohn's Disease</th>
<th>Infectious Colitis/Acute Self-limited Colitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse change</td>
<td>Yes</td>
<td>Yes</td>
<td>Sometimes</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Focal change</td>
<td>Never</td>
<td>Sometimes</td>
<td>Usually</td>
<td>Usually</td>
</tr>
<tr>
<td>Irregular luminal surface</td>
<td>Yes</td>
<td>Yes, focal</td>
<td>Sometimes may be focal</td>
<td>Sometimes may be focal</td>
</tr>
<tr>
<td>Crypt abscesses &amp; cryptitis</td>
<td>Yes</td>
<td>Yes, focal</td>
<td>Yes, focal</td>
<td>Yes, luminal accentuation</td>
</tr>
<tr>
<td>Mucin depletion</td>
<td>Diffuse</td>
<td>Focal</td>
<td>Usually focal</td>
<td>Usually focal</td>
</tr>
<tr>
<td>Architectural abnormality</td>
<td>Diffuse diffuse</td>
<td>Usually</td>
<td>Usually focal</td>
<td>Usually absent</td>
</tr>
<tr>
<td>Basal plasmacytosis</td>
<td>Yes</td>
<td>Yes</td>
<td>Usually absent</td>
<td>Usually absent</td>
</tr>
<tr>
<td>Neutrophils in the lamina propria</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Usually yes</td>
</tr>
<tr>
<td>Granulomas</td>
<td>No</td>
<td>No</td>
<td>Yes up to 28%</td>
<td>Usually no</td>
</tr>
<tr>
<td>Submucosal inflammation</td>
<td>Usually no</td>
<td>Usually no</td>
<td>Sometimes</td>
<td>Usually no</td>
</tr>
</tbody>
</table>

*FROM REFERENCE 1

Antineutrophil cytoplasmic antibodies (ANCAs) have been identified in patients with inflammatory bowel disease (27-29). The immunofluorescence pattern (perinuclear) differs from the diffuse cytoplasmic...

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reaction detected in patients with Wegener's granulomatosis. Although initially thought to be specific for ulcerative colitis, ANCs probably more reflect inflammatory diseases involving the colon. In one series, 71% of patients with presumed ulcerative colitis had ANCs with a 46% prevalence in colonic Crohn's disease, and only a 5% prevalence in patients with infectious-type colitis (17). Recently, investigators have focused on anti-Saccharomyces cerevisiae antibodies (ASCA) in Crohn’s disease. ASCA can be identified in 60-70% of patients with Crohn’s disease versus 10-15% in ulcerative colitis and in 0-5% of controls (30,31). Some have used the combination of p-ANCA and ASCA to increase specificity (32). These tests clinically have demonstrated low sensitivity of 50-60% but have been reported to be highly (up to 100%) disease specific. These tests may have value in classification of indeterminate colitis but this remains to be proved.

**Infectious colitis/acute self-limited colitis**

The following histologic appearances have been described in culture-proved infectious colitis (1); normal colon, nonspecific increases in chronic inflammatory cells, diffuse active colitis (20), ischemic-like changes (33,34), and the focal active colitis pattern of injury. Although colonic mucosal biopsy appearance in infectious colitis can vary greatly, a large number will demonstrate the focal active colitis pattern. In general, invasive organisms cause greater changes in morphology than those producing their effect by toxins.

Histological evaluation, though helpful in suggesting an infectious etiology, can only rarely suggest a specific etiology. The definitive diagnosis of infectious colitis requires laboratory recovery of the offending organism or demonstration of a fourfold rise is a specific antibody titer.

There remain, even after extensive microbiologic work-up, a subset of patients presumed clinically to have infectious colitis, who experience spontaneous recovery in less than six months, in whom biopsy specimens demonstrate focal active colitis and no infectious etiology can be identified. The term “acute self-limited colitis” has been used to describe such patients (15-17,19). I prefer the term “infectious-type colitis” over "acute self-limited colitis" because some examples of "acute self-limited colitis" may not be self-limited (8). Others prefer the term “non-relapsing” colitis (17,18).

**Colonic infections causing ischemic-like histologic changes**

*Clostridium difficile-associated Colitis*

Administration of any antibiotic that favors the growth of toxin-producing C. difficile can lead to pseudomembranous colitis. Early studies linked Clindamycin and Lincomycin to pseudomembranous colitis, but in terms of absolute numbers, the vast majority of cases are linked to ampicillin, penicillin, and the cephalosporins because of their far more prevalent use (35). Pseudomembranous colitis has even been associated with antineoplastic chemotherapeutic agents that have antimicrobial activity (36).

Symptoms of C. difficile-associated colitis usually develop during the administration of antibiotics but in up to one-third of patients, the onset of symptoms can be delayed up to four to six weeks (33,37-40). The characteristic endoscopic and histologic pseudomembrane lesion is found only early in the course of the disease. The surface of the mucosa is covered by a plaque-like cream to yellow-colored pseudomembrane (33). The intervening mucosa frequently appears normal but can be hyperemic or edematous. With increasing severity, the membranes can become confluent and linear ulcers can develop.

Histologically, there is a patchy necrosis of the superficial colonic crypts that can be similar to ischemia (33,41). The affected crypts become dilated near the surface and an inflammatory exudate erupts from the...
superficial aspect of the degenerating crypts in an explosive or mushroom-like configuration. The pseudomembrane may cover adjacent virtually normal colonic mucosa. The karyorrhectic debris and neutrophils in the pseudomembranes tend to orient in a curious linear fashion within the fibrin and mucin. Many examples of C. difficile-associated colitis and occasionally the mucosa between diagnostic pseudomembranes can show the focal active colitis pattern of injury described above (15,16). Left untreated, some cases of C. difficile-associated colitis may progress and can become indistinguishable from ischemic bowel disease. Toxic megacolon and perforation can occur.

**Enterohemorrhagic Escherichia coli (EHEC)-associated Colitis (Hemorrhagic Colitis)**

The clinical syndrome of "hemorrhagic colitis" is characterized by abdominal cramping, bloody diarrhea, and no or low-grade fever (34,42). Patients typically demonstrate right-sided colonic edema, erosion, and hemorrhage (34,43) and the absence of "conventional" enteric pathogens. In 1983, investigation of hemorrhagic colitis outbreaks occurring in Oregon and Michigan implicated a then-rare serotype of *Escherichia coli*, O157:H7, as the cause of the syndrome (44).

Subsequently, investigations of several additional outbreaks have confirmed the association between hemorrhagic colitis and the verocytotoxin-producing *E. coli*, the most important of which is *E. coli* O157:H7 (45,46).

Patients with hemorrhagic colitis typically present with sudden onset of crampy abdominal pain occurring three to four days after ingestion of contaminated food, usually undercooked hamburger. Outbreaks have also been linked to other foods, drinking water, and swimming pools (47). Watery diarrhea, nausea, and vomiting follow within hours. One to two days later, grossly bloody diarrhea replaces the watery diarrhea. In almost all patients, the disease resolves spontaneously, usually within eight days. Investigation of the epidemic outbreaks of *E. coli* O157:H7 infection has revealed that not all patients acquire the full syndrome of hemorrhagic colitis. Rather, a clinical spectrum exists ranging from asymptomatic carrier or self-limited non-bloody diarrhea to severe cases complicated by hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (45,46,48,49).

*E. coli* O157:H7 produces several toxins active against vero cells (verocytotoxins) and HeLa cells that have been termed Shiga-like toxins because their mode of action is similar to that of the toxin produced by *Shigella dysenteriae* type 1. The toxins interact with the membrane receptor-globotriosyl ceramide, are apparently absorbed into epithelial and endothelial cells, and cause damage or cell death by interfering with protein synthesis (45,50).

Colonic histology in EHEC infection is by far the best documented of the diarrheagenic *E. coli* because EHEC infection can lead to hospitalization and it clinically mimics ischemia and primary inflammatory bowel disease, thus prompting colonoscopy (34,51-53). Colonoscopy typically demonstrates patchy erythema, edema, and surface ulceration of the colon. The cecum and ascending colon are usually described as markedly abnormal while the left colon typically had mild or no changes. Histologically, specimens usually show hemorrhage and edema within the lamina propria. Specimens most often show focal necrosis associated with hemorrhage and acute inflammation within the superficial mucosa and preservation of the deep colonic crypts, similar to the pattern of injury described with acute ischemic colitis. Specimens from many patients show neutrophils focally infiltrating the lamina propria and crypts resembling the focal active colitis pattern of injury seen in infectious colitis/acute self-limited colitis (15,16,18). Rarely patients also demonstrate poorly formed inflammatory pseudomembranes. This combination of ischemic-like and infectious-like injury with capillary thrombi should at least suggest verocytotoxin-producing *E. coli*-associated colitis.

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Routine stool culture media will not distinguish \textit{E. coli} O157:H7 from other strains of \textit{E. coli} normally present in the stool. Physicians suspecting hemorrhagic colitis caused by \textit{E. coli} O157:H7 should specifically request that stools be screened for this organism. Specimens may be examined using sorbitol fermentation as a strain marker; unlike most \textit{E. coli}, \textit{E. coli} O157:H7 strains test sorbitol negative or delayed positive (54). Colonies that are sorbitol negative at 24 hours can be screened with commercial O157 antisera. Additional biochemical tests and H-antigen determinations can be performed at a later time (34). DNA hybridization techniques, PCR direct immunofluorescence and latex agglutination techniques have also been described for identification. Procedures for detection of free fecal verocytotoxin and more sensitive methods for screening stool cultures for verotoxin-producing \textit{E. coli} using Polymyxin B on colony sweeps have been reported (45-47).

\textbf{Other E-coli Pathogens}

\textit{Escherichia coli} are a predominant component of the gut microflora. Although the vast majority are harmless or even beneficial, at least six major categories of \textit{E. coli} intestinal pathogens are recognized (Table 3) (55,56).

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|}
\hline
\textbf{Category} & \textbf{Virulence Mechanisms} & \textbf{Clinical Features} \\
\hline
Enterotoxigenic (ETEC) & Heat labile and/or stable toxins & Watery diarrhea in travelers and children \\
& Adherence & \\

Enteroinvasive (EIEC) & Adherence and invasion & Dysentery \\

Enterohemorrhagic (EHEC) & Shiga-like toxins & Bloody diarrhea \\
& Adherence & HUS/TTP \\

Enteropathogenic (EPEC) & Attachment and effacement & Watery diarrhea in children \\

Enteroaggregative (EAEC) & Adherence, ? cytotoxin & Watery or persistent diarrhea \\
& & Children/developing countries, traveler’s diarrhea, diarrhea in HIV patients \\

Diffusely Adherent (DAEC) & Adherence & Acute or persistent diarrhea \\
& & Children/developing countries \\
\hline
\end{tabular}
\caption{\textit{E. coli} Intestinal Pathogens}
\end{table}

A great deal is known about the microbiology, pathogenic mechanisms, virulence factors, molecular genetics, and epidemiology of intestinal \textit{E. coli} infections. However, surprisingly little is known about the histopathology of the human gut infection. Information remains scant because most infectious diarrheas, being self-limited, do not require specific treatment even when an infectious organism is identified. Therefore, sophisticated diagnostic tests such as organism identification, virulence factor determination, and endoscopy with biopsy, are reserved for outbreaks or for cases with unusual features (e.g., severe or protracted diarrhea, systemic symptoms, patients requiring hospitalization, or patients in which the differential diagnosis includes other serious diseases such as inflammatory bowel disease or ischemia).

Analysis of the information available yields a limited number of reaction patterns in \textit{E. coli}-associated infection that have been described in human gut or inferred from in vitro models or from human infection with other organisms known to have similar virulence factors. These include: no histologic change.
ETEC), observation of surface adherent organisms (EPEC, EAEC, ? DAEC), mild non-specific inflammation (? all subtypes), ischemic-like change (EHEC), and acute infectious (self-limited) colitis (EHEC, EIEC, ? ETEC).

Enterotoxigenic \textit{E. coli} (ETEC) are thought to adhere to and colonize the surface of the small bowel where they elaborate their toxins (55). It is possible that a serendipitously obtained small bowel biopsy specimen may contain adherent surface bacteria. Histological appearances of small bowel and colon are inferred from ETEC’s close relationship to \textit{Vibrio cholera}, which does not cause histologically recognizable lesions in the small bowel or the colon. Therefore, normal colon would be expected in ETEC-associated diarrhea. It is possible that ETEC could cause some acute self-limited colitis.

Recognizing the difficulty in identifying pathogenic \textit{E. coli} in routine stool culture, it is possible that EIEC and EHEC could likewise be responsible for some acute self-limited colitis.

As stated above, the diffuse active colitis pattern can also be seen in some examples of Crohn's colitis (11), and in some cases of documented infectious colitis (12). These infectious colitis cases were associated with an epidemic outbreak of \textit{Shigella} dysentery. Since the pathogenic features of \textit{Shigella} species are virtually identical to EIEC (1), it is possible that EIEC may on rare occasions cause the diffuse active colitis pattern of injury.

The ability to adhere and to colonize host enterocytes and/or colonocytes is requisite for human infection for all six recognized categories of diarrheogenic \textit{E. coli}. However, EPEC, EAEC, DAEC, which are traditionally referred to as enteroadherent \textit{E. coli}, are best differentiated on the basis of their adherence patterns to HEp-2 cells in culture. These patterns include localized adherence (EPEC), aggregative adherence (EAEC), and diffuse adherence (DAEC) (55).

The human gut histopathology in EPEC in vivo has been described (57,58) and is similar to that seen in experimental animal models (59). Jejunal biopsy specimens have demonstrated a variable villous abnormality without acute inflammation (57,58). Adherent bacteria could be identified with routine H & E stained sections on the luminal surface. Adherent surface bacteria have also been seen in colorectal biopsy specimens (57). The characteristic attaching and effacing lesion can only be recognized by the use of electron microscopy (55,57,58). The bacteria intimately adhere to the host cell by an attachment pedestal and cause effacement of the adjacent microvilli.

Human histopathology associated with EAEC and DAEC infection can only be inferred from animal models and in vitro studies (55,60–62). Presumably, bacteria adhere to the surface epithelium of the small intestine and the colon (62a). EAEC and DAEC infection may be associated with a variable villous lesion. Electron microscopy has demonstrated adherent bacteria in cell culture with normal microvillous architecture. In the colon, EAEC produces cytotoxic effects ultrastructurally (microvillous vesiculation, enlarged crypt openings, creation of intercrypt crevices and mucosal epithelial cell extrusion (62a).

Ironically, the most detailed description of human ileal and colorectal infection with EPEC, EAEC, and possibly DAEC resides in a review of 52 patients with Acquired Immunodeficiency Syndrome reported by Ornstein and Kotler (63). Unfortunately, the exquisite light and electron microscopic descriptions of these diarrheogenic bacterial enterocolitides were not complimented by sophisticated microbiological studies. Therefore, identification of these organisms as \textit{E. coli} rests on the pathologic similarity to other reported cases and preliminary isolation studies that have shown that at least some of these cases were EAEC and DAEC (63).
The colonic histology showed surface epithelial degeneration with adherent bacteria (some being extremely subtle) without colonic architectural distortion or significant inflammation. Electron microscopy demonstrated three patterns: a) typical adhering and effacing lesions (like EPEC), b) a loosely adherent pattern with effacement (like EAEC), and c) an intercalated pattern with effacement in which vertically oriented bacteria were seen burrowed between intact microvilli. The intercalated pattern could be DAEC in vivo because it is similar to the pictures and descriptions of DAEC in other in vitro and animal model systems (62).

**Acute diarrhea with colonic epithelial lymphocytosis -Brainerd Diarrhea**

The term Brainerd Diarrhea has been applied to outbreaks of diarrhea of unknown etiology characterized by acute onset and prolonged duration (64). The disease was named after Brainerd, Minnesota where in 1984, 122 residents developed watery diarrhea after drinking unpasteurized milk from a local dairy. A second outbreak occurred in Henderson County, Illinois in 1987, when 72 people developed watery diarrhea associated with drinking contaminated well water at a roadside restaurant. In each outbreak, patients underwent extensive diagnostic evaluations including comprehensive microbiological studies of their stool and the implicated exposure site. Despite this work up, no etiologic agent was identified. The clinical and epidemiologic characteristics were typical for a point-source epidemic infectious diarrhea. However, unlike typical infectious diarrhea, these patients developed a chronic watery diarrhea syndrome with symptoms lasting longer than six months and often lasting for years.

We examined colonic and small bowel biopsy specimens from 22 patients who were involved in an outbreak of Brainerd Diarrhea that was linked to the water supply of a cruise ship visiting the Galapagos Islands. In general, the small bowel biopsy specimens were histologically normal. Colonic biopsy specimens from 20 patients revealed surface epithelial lymphocytosis without distortion of mucosal architecture, surface degenerative changes or thickened subepithelial collagen plate. The degree of surface epithelial lymphocytosis was similar to that seen in collagenous colitis and lymphocytic colitis (64,65,66). We also evaluated a limited number of colonic biopsy specimens from two other outbreaks of Brainerd Diarrhea. They revealed similar histologic findings to the Galapagos outbreak.

We now believe that patients with the clinical syndrome of chronic watery diarrhea of unknown etiology and patients reported as having lymphocytic colitis represent a heterogeneous group that may contain persons with unrecognized Brainerd Diarrhea. With longer-term follow-up, the “Brainerd” cases appear to be self-limited with patients recovering in less than 3 years. From a practical standpoint, we believe surface epithelial lymphocyte counts should be performed on all colonic biopsy specimens from patients with chronic diarrhea, especially chronic watery diarrhea. Somewhat lower lymphocyte counts and a lesser degree of surface degeneration may distinguish Brainerd Diarrhea from other types of “lymphocytic colitis” but more study is required. Currently, Brainerd Diarrhea cannot be recognized outside the setting of an epidemic.

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CHRONIC WATERY DIARRHEA AND EPITHELIAL LYMPHOCYTOSIS: THE “KINDER, GENTLER” COLITIDES

Collagenous Colitis

Collagenous colitis, first recognized by Lindstrom in 1976 (1), describes a distinct clinicopathologic syndrome causing watery diarrhea, predominantly in middle-aged or older (mean age = 59 years) women (male:female = 1:7.5) (2-4). Colonoscopic and barium enema examination in these patients are usually normal. Therefore, diagnosis depends upon recognition of characteristic changes in biopsy specimens. The primary histologic change of collagenous colitis consists of a patchy increase in the thickness of the subepithelial collagenous plate (2,5). The normal colonic subepithelial collagen layer measures approximately 5 um in thickness, but in collagenous colitis it may increase to 10 um or more (approximately the diameter of two red blood cells). The collagen had been identified mostly as types I and III but type VI collagen has also been described (6). Tenascin has been identified in the subepithelial collagen layer and can be exploited diagnostically by immunohistochemistry (6a). A mild to moderate increase in chronic inflammatory cells including plasma cells expands the lamina propria. Patchy injury to the surface epithelium characterized by increased numbers of intraepithelial lymphocytes, epithelial degeneration, and sloughing also occurs. Atrophy, mucosal architectural distortion, and acute inflammation are usually not present or are minimal (2,5,7).

Differential diagnostic considerations include nonspecific changes, primary inflammatory bowel disease, acute colitis, mucosal prolapse syndrome (solitary rectal ulcer syndrome), ischemic bowel disease, amyloidosis, and lymphocytic ("microscopic") colitis. Features comparing and contrasting the remaining conditions in the differential diagnosis are listed in the Table.

Lymphocytic ("Microscopic") Colitis

Read and colleagues introduced the term "microscopic colitis" to describe patients with chronic watery diarrhea of unknown origin occurring in middle-aged (mean = 54 years) patients (8). Like collagenous colitis, the colonoscopic appearance and the barium enema were usually described as normal. Biopsy specimens demonstrated increased inflammatory cells in a pattern not specific for any established entity (8). Since this initial report, several additional investigators have refined the clinical and especially the histologic diagnostic criteria (7,9,10). The histologic changes include increased chronic inflammatory cells in the lamina propria and surface epithelium, degenerative changes of the surface epithelium, minimal architectural changes, and minimal acute inflammation. The changes of "microscopic colitis" in general resemble the surface epithelial and lamina propria changes of collagenous colitis, and indeed changes identical to "microscopic colitis" are seen in biopsy specimens from patients with collagenous colitis if areas where the collagen plate is not well developed are sampled. Because of the lymphocyte predominance in the inflammatory infiltrate, Lazenby et al. (7) proposed the term "lymphocytic colitis" to describe this entity. They cited that the term "microscopic colitis" could be confused with other diseases that can have a normal gross appearance and an abnormal histology (e.g., Crohn's disease, acute self-limited colitis).

The histologic differential diagnosis of lymphocytic colitis includes changes caused by enema (bowel preparation), the mucosal M cell zone, acute colitis, primary inflammatory bowel disease, and collagenous colitis. Features comparing and contrasting these various conditions are also included in the table.

Collagenous and Lymphocytic Colitis: Different Names for the Same Condition?
The similarities between lymphocytic colitis and collagenous colitis are striking. They share similar symptomatology and endoscopic findings. The histologic similarities include the increased intraepithelial lymphocytes, the surface epithelial damage, and the increased chronic inflammatory cells within the lamina propria (5,7,11,12). Both collagenous and lymphocytic colitis can be associated with other autoimmune phenomenon such as thyroid disease, enteropathic arthritis, rheumatoid arthritis, myasthenia gravis and celiac sprue (13-15). One patient with well established microscopic colitis developed collagenous colitis three years after the initial diagnosis (7). Some cases of lymphocytic colitis demonstrate some thickening of the subepithelial collagen plate and the distinction of lymphocytic colitis from collagenous colitis may not be so clear cut in all cases (12). It appears likely that lymphocytic colitis and collagenous colitis are either the same or very similar entities, perhaps representing different morphologic phases of one disease process (7,11,13).

There are some minor differences between lymphocytic colitis and collagenous colitis that deserved mention. Collagenous colitis has been reported predominantly in women, whereas lymphocytic colitis affects men and women equally (7,16). However, the Mayo Clinic reported a similar frequency of collagenous colitis in men and women (14). Giardiello et al have reported differences in HLA haplotypes between collagenous colitis and lymphocytic colitis with lymphocytic colitis having a higher frequency of HLA A1 and a lower frequency of HLA A3 (16), but the number of observations was quite small. Finally, collagenous colitis and lymphocytic colitis do differ histologically with the presence of the collagen plate in the former.

**Pathogenesis of the Diarrhea**

Since lymphocytic and collagenous colitis share so many features, most clinical reviews have included them both together. In both conditions there appears to be net fluid secretion in the colon that is primarily responsible for the diarrhea (11,18). Several investigators could find no association between the thickness and extent of the collagen plate and the amount of diarrhea (12,14). Therefore, most investigators conclude that it is the damage to the surface epithelial cells and the inflammation rather than the collagen deposits that causes the diarrhea (12). The presence and thickness of the subepithelial collagen plate appears unrelated to the patient age or duration of disease (12). Interestingly, a minority of patients have also demonstrated evidence for small bowel dysfunction with salt wasting, fatty acid malabsorption, small bowel net secretion, and rarely a small bowel villous lesion that resembles celiac sprue (see below) (17).

**Treatment and Prognosis**

Spontaneous resolution of collagenous colitis and lymphocytic colitis has occurred, thus rendering evaluation of therapeutic regimens difficult (19,19a). A distinct minority of patients with these colitides have presented with relatively mild diarrhea and have achieved medical control with dietary restriction, (elimination of caffeine and lactose containing foods) bulking agents, and antimotility drugs (loperamide hydrochloride, diphenoxylate hydrochloride, atropine) (17,19). This type of "symptomatic" therapy has failed, however, in the vast majority of patients thus necessitating the addition of anti-inflammatory agents. Approximately half of the patients with lymphocytic and collagenous colitis respond to Sulfasalazine and other 5-ASA products alone with diarrhea subsiding in one to two weeks. In non-responders or in those intolerant of Sulfasalazine, Prednisone can be used either with or instead of Sulfasalazine. Approximately 80-90% of patients eventually respond to this approach (3,11,17,19,19a,20). Treatment of the often-associated thyroid problem can also improve bowel function. There are also case reports of improvement with Pepto-Bismol, mepacrine hydrochloride, steroid enemas, metronidazole, and 5-aminosalicylate (11,17,19a,20).
Etiology and Pathogenesis

NSAIDS and ticlopidine have been reportedly linked to some occurrences of collagenous colitis (21,21a), and lymphocytic colitis has been reported in patients receiving the drug Cyclo 3 Fort (22) ranitidine (23), ticlopidine and flutamide (21a). However, most cases cannot be linked to drug ingestion and the bulk of the evidence suggests that lymphocytic colitis and collagenous colitis share a common immune-mediated etiology and pathogenesis. Both conditions have a striking histologic similarity to celiac sprue, a condition known to be autoimmune possibly having a viral or infectious trigger (7). In addition, both lymphocytic and collagenous colitis have been linked to other conditions thought to have autoimmune pathogenesis such as Crohn’s disease, hypothyroidism, hyperthyroidism, inflammatory arthropathies, pernicious anemia, small bowel villous atrophy, iritis, and myasthenia gravis (13,14,18,19,23a). Finally, both conditions respond dramatically to anti-inflammatory agents (17,19). Recently, a "lymphocytic colitis-like" histology was reported in an epidemic outbreak of Brainerd Diarrhea linked to a water tank aboard a cruise ship supporting an infectious trigger for some cases of lymphocytic colitis. (See below)

Association with Celiac Sprue

The association between lymphocytic colitis/collagenous colitis and celiac sprue and "sprue-like" lesions deserves special attention (24-26). In the experience of DuBois and associates (25) and Wolber and colleagues (26), approximately 25% of patients with "celiac sprue" who had colonic biopsies also showed changes of lymphocytic colitis. Similarly, approximately 15% of patients with lymphocytic colitis who also had small bowel biopsy showed sprue-like histology (26a). Colonic microscopic abnormalities in patients with celiac sprue occur after experimental exposure to wheat or gliadin enemas (25-28) suggesting that the entire intestinal tract may be susceptible to gluten-induced injury. It is possible that in some patients with true celiac sprue (responsive to gluten withdrawal), occult dietary gluten actually reaches the colon and induces the histologic changes of "lymphocytic colitis." However, approximately one-half of the patients with "sprue-like" small bowel lesions and lymphocytic colitis have not responded to gluten withdrawal. The term "lymphocytic enterocolitis" has been coined to describe this refractory sprue-like condition associated with colonic abnormalities (25).

Aberrant Histology in Lymphocytic and Collagenous Colitis

As more cases are investigated, it is not unusual to see variations in histology comimgled with the classic changes of lymphocytic or collagenous colitis. These variations include architectural change, cryptitis, Paneth cell metaplasia, ulcers and inflammatory membranes (28a, 28b, 28c, 28d). Approximately 1/3 of patients demonstrate cryptitis. Rarely one encounters inflammatory membranes. Neither histology correlates with infection. Approximately 2% of patients have ulcers with architectural abnormalities reported in 5%, Paneth cell metaplasia have been seen in 14-44% of patients, more often seen in collagenous colitis where it correlates with increased severity of disease. Acute inflammation was seen in increased frequency in patients taking antibiotics. Ulcers are linked with concomittant NSAID usage. Aberrant histology does not seem to correlate with symptoms, results of medical treatment or outcome. No patient with unusual histology, including those with architectural changse and Paneth cell metaplasia, has yet to be developed primary inflammatory bowel disease.

Brainerd Diarrhea

The term Brainerd Diarrhea has been applied to outbreaks of diarrhea of unknown etiology characterized by acute onset and prolonged duration (29). The disease was named after Brainerd, Minnesota, where in 1984, 122 residents developed watery diarrhea after drinking unpasteurized milk from a local dairy. A second outbreak occurred in Henderson County, Illinois in 1987, when 72 people developed watery
diarrhea associated with drinking contaminated well water at a roadside restaurant. In each outbreak, patients underwent extensive diagnostic evaluations including comprehensive microbiological studies of their stool and the implicated exposure site. Despite this work up, no etiologic agent was identified. The clinical and epidemiologic characteristics were typical for a point-source epidemic infectious diarrhea. However, unlike typical infectious diarrhea, these patients developed a chronic watery diarrhea syndrome with symptoms lasting longer than six months and often lasting for years.

We examined colonic and small bowel biopsy specimens from 22 patients who were involved in an outbreak of Brainerd Diarrhea that was linked to the water supply of a cruise ship visiting the Galapagos Islands. In general, the small bowel biopsy specimens were histologically normal. Colonic biopsy specimens from 20 patients revealed surface epithelial lymphocytosis without distortion of mucosal architecture, surface degenerative changes or thickened subepithelial collagen plate. The degree of surface epithelial lymphocytosis was similar to that seen in collagenous colitis and lymphocytic colitis (7,29,30). We also evaluated a limited number of colonic biopsy specimens from two other outbreaks of Brainerd Diarrhea. They revealed similar histologic findings to the Galapagos outbreak.

We now believe that patients with the clinical syndrome of chronic watery diarrhea of unknown etiology and patients reported as having lymphocytic colitis represent a heterogeneous group that may contain persons with unrecognized Brainerd Diarrhea. With longer-term follow-up, the “Brainerd” cases appear to be self-limited with patients recovering in less than 3 years. From a practical standpoint, we believe surface epithelial lymphocyte counts should be performed on all colonic biopsy specimens from patients with chronic diarrhea, especially chronic watery diarrhea. Somewhat lower lymphocyte counts and a lesser degree of surface degeneration may distinguish Brainerd Diarrhea from other types of “lymphocytic colitis” but more study is required. Currently, Brainerd Diarrhea cannot be recognized outside the setting of an epidemic.
**TABLE**

**COLLAGENOUS AND LYMPHOCYTIC COLITIS: DIFFERENTIAL DIAGNOSIS**

<table>
<thead>
<tr>
<th>Colitis</th>
<th>Lymphocytic Colitis</th>
<th>Collagenous Colitis</th>
<th>IBD</th>
<th>Acute Colitis</th>
<th>SRUS</th>
<th>Ischemia</th>
<th>Amyloid</th>
<th>Enema</th>
<th>M-cell Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraepithelial lymphocytes</td>
<td>+++</td>
<td>+++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+++*</td>
</tr>
<tr>
<td>Lamina propria Chronic inflammation</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+++*</td>
</tr>
<tr>
<td>Surface PMN's</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>±</td>
<td>0</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+**</td>
<td>0</td>
</tr>
<tr>
<td>Lamina propria PMN's</td>
<td>±</td>
<td>±</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cryptitis/ crypt abscesses</td>
<td>±</td>
<td>±</td>
<td>+++</td>
<td>++</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Surface epithelial degeneration</td>
<td>++</td>
<td>+++</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>±</td>
<td>0</td>
</tr>
<tr>
<td>Mucosal architectural distortion</td>
<td>±</td>
<td>±</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Collagen deposits</td>
<td>0</td>
<td>Yes#</td>
<td>0</td>
<td>0</td>
<td>Yes##</td>
<td>Yes##</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Positive amyloid stain</td>
<td>0</td>
<td>0</td>
<td>±</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+++</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Over lymphoid aggregate only  ** Surface only

IBD = Primary inflammatory bowel disease
SRUS = Solitary rectal ulcer syndrome

# Subepithelial
## Throughout lamina propria

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REFERENCES


EARLY COLORECTAL CARCINOMA

Robert E. Petras, M.D.
Adrian Ormsby, M.D.

Adenomas and Malignant Colorectal Polyps: Nomenclature

The nomenclature of colorectal adenomas, dysplasia and malignant polyps can be confusing. Unfortunately, there is no universally accepted nomenclature (1). Most surgical pathologists appear to apply variations of the 1989 World Health Organization (WHO) terminology (2). According to this system, the terms dysplasia, adenocarcinoma in-situ, intramucosal adenocarcinoma and invasive adenocarcinoma are acceptable and each has a precise meaning when applied to colorectal polyps. Appropriate patient care requires that the endoscopist, surgeon and surgical pathologist understand the significance of each of these terms.

I have used the following criteria, which are based on the 1989 WHO. All adenomas have at least low-grade epithelial dysplasia. Without dysplasia, adenoma could not be distinguished from normal colonic mucosa. Low-grade dysplasia is characterized by a slight decrease in the amount of intracellular mucin, mild nuclear enlargement with hyperchromasia with some nuclear stratification, and an increased number of mitoses figures. With increasing degrees of dysplasia (low-grade to high-grade) there is progressive loss of intracellular mucin, progressive nuclear enlargement with stratification and a loss of nuclear polarity. Adenocarcinoma in-situ describes the next step in the dysplasia-carcinoma sequence. Here, the atypical glands assume a complex cribriform or back-to-back gland configuration but the basement membrane remains intact. Some consider carcinoma in-situ as part of the spectrum of high-grade dysplasia and report both under the same term (3). When carcinoma cells infiltrate into the lamina propria and/or muscularis mucosae only, terms such as high-grade dysplasia and carcinoma in-situ are technically no longer accurate (because both require an intact basement membrane) and the term intramucosal adenocarcinoma may be used (2). Finally, when carcinoma cells have invaded the submucosa (or beyond) the lesion is labeled invasive adenocarcinoma. Invasion of the submucosa is invariably associated with an infiltrative pattern to neoplastic glands associated with tumor desmoplasia. This tumor desmoplasia is extremely helpful in recognizing invasion (defined as infiltration of at least the submucosa) especially in small biopsy specimens.

The nomenclature controversy principally centers on the observation that in the colon, infiltrating carcinoma cells do not become clinically significant (i.e. able to metastasize) until they have invaded the submucosa (4,5). Only polyps containing invasive adenocarcinoma require a therapeutic decision on the part of the clinician. Adenoma (adenoma with dysplasia), adenocarcinoma in-situ and even intramucosal adenocarcinoma lack metastatic capability and are adequately treated by polypectomy alone (5). As a result, some pathologists advocate modification of the nomenclature to account for clinical behavior (1). Although the 1989 WHO accepted and defined two (low-grade, high-grade) or three grades (mild, moderate, severe) of dysplasia, carcinoma in-situ and intramucosal carcinoma, they recommended a similar behavior-based modification for intramucosal carcinoma stating; “… intramucosal adenocarcinoma of the colon has not been shown to metastasize, and for this reason ‘carcinoma in-situ’ is more appropriate. To prevent potential confusion, the term ‘intramucosal carcinoma’ is best avoided in the large bowel” (2).

The 2000 version of the WHO classification added little clarification and introduced additional and in my opinion, confusing terminology. The authors state that the defining feature for colorectal adenocarcinoma is invasion through the muscularis mucosae into the submucosa. This is something all can agree upon.
However, once adenocarcinoma has been so defined, worrisome lesions not fulfilling the criteria for adenocarcinoma become difficult to describe. For example, the WHO attempts to define adenocarcinoma in-situ and intramucosal adenocarcinoma as lesions with morphologic characteristics of “adenocarcinoma” confined to the epithelium or that “invade” the lamina propria alone and lack invasion through the muscularis mucosae. The WHO goes on to state that these lesions have virtually no risk of metastasis. According to the WHO, the term “... high-grade intraepithelial neoplasia is more appropriate than adenocarcinoma in-situ and ... intramucosal neoplasia is more appropriate than intramucosal adenocarcinoma”. The WHO believes that use of these terms will help avoid overtreatment. The problems I have with this classification are many. The loose use of the term “invasion” to describe lesions that are not by definition invasive adenocarcinoma is confusing. The lesser lesion of high-grade intraepithelial neoplasia sounds worse than the term used to describe intramucosal adenocarcinoma (intramucosal neoplasia). Furthermore, all adenomas, strictly speaking are intraepithelial or intramucosal neoplasms.

The latest effort to reach consensus (largely between Eastern [Japanese] and Western Pathologists) (7,8,9) resulted in the Vienna classification of the gastrointestinal neoplasia (7) presented in Table 1.

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative for neoplasia/dysplasia</td>
</tr>
<tr>
<td>2</td>
<td>Indefinite for neoplasia/dysplasia</td>
</tr>
<tr>
<td>3</td>
<td>Non-invasive low-grade neoplasia (low grade adenoma/dysplasia)</td>
</tr>
</tbody>
</table>
| 4 | Non-invasive high-grade neoplasia  
- High grade adenoma/dysplasia  
- Non-invasive carcinoma (CIS)  
- Suspicious for invasive carcinoma |
| 5 | Invasive neoplasia  
- Intramucosal carcinoma  
- Submucosal carcinoma or beyond |

Problems with the Vienna system include: a) the loose application of the word invasion, b) category IV “non-invasive” high-grade neoplasia including potentially dangerous lesions (suspicious for invasive adenocarcinoma) and: c) Category V “invasive neoplasms” including intramucosal adenocarcinoma which is known to be clinically benign in the colon and rectum. It is unlikely that a numerical system without clinical correlation will ever gain widespread acceptance.
A Biased and Pragmatic View on Nomenclature and Reporting

As modified from the 1989 WHO classification described above, low-grade dysplasia, high-grade dysplasia, adenocarcinoma in-situ, intramucosal adenocarcinoma exist and can be recognized by pathologists. This nomenclature is attractive because it can be applied throughout the entire gastrointestinal tract. If one chooses to diagnose high-grade dysplasia, adenocarcinoma in-situ, and intramucosal adenocarcinoma in colorectal biopsy specimens, I specifically mention that these lesions lack metastatic potential in the report.

Since infiltrating carcinoma cells in a colorectal polyp do not become clinically significant (i.e., able to metastasize) until they have invaded the submucosa (4,5,11-38), only a polyp containing invasive adenocarcinoma (invasion of at least the submucosa) should be considered malignant. Only invasive adenocarcinoma requires a therapeutic decision. Therefore the presence or absence of invasive adenocarcinoma should be specifically mentioned in the pathology report.

Mistakes pathologists make in reporting colorectal adenoma, dysplasia, and malignant polyps occur in three major categories: 1) the pathology report is not clear. (Nonspecific or noncommittal terms are used or the presence or absence of invasion is not clearly stated); 2) mispositioned glands (pseudocarcinomatous invasion) are misinterpreted as invasive carcinoma. 3) the margin of excision is either not identified or not commented upon.

Differential Diagnosis

A common problem concerns differentiating invasive carcinoma in a colorectal polyp from pseudocarcinomatous invasion. Pseudocarcinomatous invasion describes a situation in which neoplastic glands of the adenoma have been mispositioned, presumably by trauma, into or beneath the muscularis mucosae (39-47). Pseudocarcinomatous invasion is relatively common, having been reported in 3% to 10% of resected polyps (39,41,42). Distinguishing this epithelial misplacement from invasive adenocarcinoma can be difficult. Reported series of "malignant polyps" have included, and indeed even illustrated polyps with pseudocarcinomatous invasion as examples of invasive carcinoma associated with adenoma (40). Histological features favoring pseudoinvasion include; lack of an infiltrative pattern, lack of tumor desmoplasia, presence of lamina propria around the mispositioned glands, lack of increased atypia in mispositioned epithelium as compared to the surface epithelium of the adenoma, and the presence of hemorrhage and/or hemosiderin deposits in nearby connective tissue.

Occasionally, the misplaced glands of pseudocarcinomatous invasion can become cystic and can be associated with dissection of mucus into the connective tissue of the polyp stalk. Here the distinction between mucinous adenocarcinoma and misplaced glands can be difficult (39-48). Table 2 illustrates histological features that aid in this differential. Remember that examination of additional sections can help in difficult cases because almost all mucinous adenocarcinomas contain foci of typical nonmucinous-type adenocarcinoma.
TABLE 2 - DIFFERENTIAL FEATURES BETWEEN DISSECTING MUCUS OF PSEUDOCARCINOMATOUS INVASION & INVASIVE MUCINOUS ADENOCARCINOMA

<table>
<thead>
<tr>
<th>Feature</th>
<th>Pseudoinvasion</th>
<th>Invasive Mucinous Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape of mucous pools</td>
<td>Rounded</td>
<td>Irregular, infiltrating</td>
</tr>
<tr>
<td>Location of epithelium</td>
<td>Periphery of pool</td>
<td>Floating in pool</td>
</tr>
<tr>
<td>Configuration of epithelium</td>
<td>Single often discontinuous layer, basal polarity of nuclei</td>
<td>Cellular piling up, complex glandular proliferation, gland in gland configuration</td>
</tr>
<tr>
<td>Cytologic features</td>
<td>Dysplasia similar to surface adenoma</td>
<td>Atypia pronounced</td>
</tr>
<tr>
<td>Tumor desmoplasia</td>
<td>Absent</td>
<td>Usually present</td>
</tr>
<tr>
<td>Hemorrhage and hemosiderin</td>
<td>Usually present</td>
<td>Usually absent</td>
</tr>
<tr>
<td>Supporting lamina propria</td>
<td>Sometimes present</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Malignant Colorectal Polyps – Patient Management

A rational decision concerning subsequent management of a patient with an endoscopically removed malignant colorectal polyp (one containing invasive carcinoma) is based upon weighing the chances of finding residual or metastatic cancer during a follow-up surgical resection (whom do I help?) against the risk of surgical mortality and morbidity (whom do I hurt?).

Some investigators have advocated subsequent major surgical resection for all patients (49). Currently, however, almost all surgeons and gastroenterologists have embraced a more conservative approach using a number of gross and histological features as "indications" for follow-up colectomy (47). These include sessile growth (5,12), residual villous adenoma, a short stalk (less than 3 mm) (13), "stalk" invasion (14), "level 4" invasion (5, 12), lymphatic or vascular permeation (34), lack of residual adjacent adenoma (polypoid carcinoma), poor differentiation (15,16,32,34) and invasive carcinoma at or near the margin of resection (15,16,32).

The Cleveland Clinic experience (16,32) parallels that of St. Mark's Hospital in London (15,19) in that use of two features appears to identify a group of patients likely to avoid an adverse outcome (residual or metastatic carcinoma in a subsequent colectomy specimen or in five-year clinical follow-up). In these studies, the patients in the "favorable histologic group" (well or moderately differentiated adenocarcinoma with a 2 mm tumor-free margin of resection in the polypectomy specimen) experienced no adverse outcome and are considered as adequately treated by polypectomy alone. Similar therapeutic recommendations have been adopted by the American College of Gastroenterology (18,18a). Their
guidelines consider colonoscopic polypectomy definitive treatment for a patient with a malignant polyp if the following criteria are fulfilled: 1) the polyp is considered completely excised at endoscopy, 2) the specimen is properly processed in the pathology laboratory, 3) the cancer is not poorly differentiated, 4) there is no histologic evidence of vascular or lymphatic involvement, and 5) the resection margin is not involved by carcinoma.

The presence of lymphatic or venous invasion has been proposed as an indication for follow-up colectomy (5,13,17,24,25,29,34,49). However, only a few malignant polyps with these features have been reported and almost all have had positive margins, grade-III invasive carcinoma, or both (35). We believe that lymphatic/venous invasion is not a reliable criterion because the distinction of true invasion from retraction artifact is frequently difficult. Cooper et al. encountered significant interobserver variation in assessing this feature (34). Furthermore, no guidelines exist that establish the extent to which a pathologist must go to diagnose lymphatic/venous invasion (e.g., number of sections or use of immunostains). We believe that you can successfully group patients into high-risk and low-risk groups without using lymphatic or venous invasion by stratification based on margin status and grade of invasive adenocarcinoma (32).

Therapeutic recommendations are difficult to make based upon any one or two studies because of low patient numbers. Therefore, Table 3 lists the major studies of endoscopic polypectomy for malignant polyps. Note that in the "favorable histologic group" the chance of finding residual or metastatic cancer in a subsequent colon resection specimen or during the follow-up period is about one percent. Weighing this against the published operative mortality rates for colectomy, that range between 2% to 8% (28-30), it would appear that subsequent major surgery should be avoided in this "favorable histologic group" (16,32).


### TABLE 3 - ENDOSCOPICALLY REMOVED MALIGNANT COLORECTAL POLYPS: FREQUENCY OF CANCER AT SURGERY OR FOLLOW-UP

<table>
<thead>
<tr>
<th>1st Author</th>
<th># of Polyps</th>
<th>Total #</th>
<th># in the Favorable Histologic Group*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chantereau (29)</td>
<td>59</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Christie (19)</td>
<td>70</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Cooper (13)</td>
<td>56</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Cranley (16)</td>
<td>40</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Coverlizza (38)a</td>
<td>50</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Cunningham (31)</td>
<td>36</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Fried (20)</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Geraghty (17)b</td>
<td>21</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hackelsberger (35)</td>
<td>86</td>
<td>8</td>
<td>1****</td>
</tr>
<tr>
<td>Kodaira (21)</td>
<td>7</td>
<td>2</td>
<td>1**</td>
</tr>
<tr>
<td>Kyz (12)</td>
<td>42</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Langer (22)</td>
<td>23</td>
<td>6</td>
<td>1***</td>
</tr>
<tr>
<td>Moore (36)</td>
<td>54</td>
<td>5</td>
<td>1****</td>
</tr>
<tr>
<td>Morson (15)</td>
<td>60</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Netzer (33)</td>
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<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Nivatvongs (14)</td>
<td>23</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Richards (27)</td>
<td>80</td>
<td>10</td>
<td>3****</td>
</tr>
<tr>
<td>Rossini (23)</td>
<td>31</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Shatney (24)</td>
<td>28</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Speroni (26)</td>
<td>30</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Volk (32)</td>
<td>47</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Whitlow (37)</td>
<td>59</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Wolff (25)</td>
<td>46</td>
<td>8</td>
<td>2**</td>
</tr>
<tr>
<td></td>
<td>1,002</td>
<td>103 (10%)</td>
<td>10 (1%)</td>
</tr>
</tbody>
</table>

*Excision complete plus (a) cancer not close (< 2 mm) to margin, (b) not grade III.
**Assessment of resection line not stated.
***Grade and distance from margin not stated.
****Distance from margin not clearly stated and may have been less than 2 mm.
a. Some may have been reported in reference 23.
b. Report details experience with 81 malignant polyps but 60 were previously reported in Reference 15.

If a decision for subsequent colon resection is made, a cancer operation is recommended rather than a more limited procedure because cancer was the indication for surgery. In Table 3, notice that the highest chance of an adverse outcome is approximately 10%. Therefore, finding no residual carcinoma in a follow-up resection specimen is the rule rather than the exception and does not indicate an inadequate dissection by the pathologist.

**Specimen Handling and Reporting**

Since evaluation of the resection line is so critical to patient management, proper handling of the polypectomy specimen is of utmost importance (16,30,32). We advocate placing the polyp immediately...
into fixative. For polyps with a stalk, we trim on either side of the stalk as illustrated in Fig. 1. The polyp with stalk can be embedded in a block, maintaining the correct anatomic relationship. For polyps without stalks (sessile growths or those where the stalk has retracted), we look for the effects of the diathermy burn on the gross specimen. This will appear as a lighter-colored area or defect on the external surface of the polyp. We carefully trim on either side of this defect (Fig. 2). We then microscopically examine a minimum of three step-sections stained with hematoxylin and eosin from each block.

In the pathology report, the presence or absence of invasive carcinoma must clearly be stated. Tumor differentiation must be noted. The resection line must be identified and assessed, and the status of that resection line must be clearly stated in the final report.

While it is clear that there is no place for crusading zeal in advising surgery, the treating physician must individualize the decision for follow up colectomy by weighing the patient’s wishes against the estimated cancer recurrence risk and the predicted operative morbidity and mortality(30). Furthermore, advancements in laparoscopic segmental resection of the colon could drastically reduce the morbidity and mortality of operative resection, which now constitutes the major contraindication for surgery. This new surgical technique may require future reassessment of current management recommendations.


REFERENCES


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PATHOLOGIC EVALUATION OF COLORECTAL CARCINOMA

Robert E. Petras, M.D.
Adrian Ormsby, M.D.

Introduction

Histologic typing, grading and clinical pathologic staging have historically provided prognostic information in patients with colorectal carcinoma. Obviously, the surgical pathologists’ skills, knowledge, and enthusiasm determine the assessment of these prognostic variables.

Tables 1-10 illustrate how the following features adversely effect prognosis: advanced Dukes’ stage, extensive local spread, lymph node involvement, aggressive histologic type, high histologic grade, extramural venous invasion and free mesothelial surface invasion (1-4). Although much useful information is gained through these “classic” grading and staging exercises, the process is not without its problems and controversy. There is not general agreement on staging or grading and current schemes all have shortcomings (5, 6). Using our current systems, the majority of patients fall into a moderate stage, moderate grade category where the probability of survival is roughly 50/50 (7).

The College of American Pathologists (CAP) have considered and commented upon the multitude of reputed prognostic factors in a consensus statement published in 2000 (8). They concluded that there were factors definitively proven to be of prognostic import including: local extent of tumor (pT); regional lymph node metastases (pN); blood or lymphatic vessel invasion; and residual tumor following surgery with curative intent (8). There were additional factors that have repeatedly been shown to be of prognostic importance. The CAP recommend that they be included in pathology reports (tumor grade, radial margin status and residual tumor in specimens following neoadjuvant therapy). Conversely, they have concluded that parameters such as tumor size and gross configuration have been well studied and are of no prognostic significance. That still leaves an incredibly large group of factors that are considered promising but have not yet been sufficiently studied. These factors are listed in Tables 11-14.

This discussion will focus on controversial areas in classic staging and grading. These include methods of lymph node dissection and assessment of histologic grade and type. Additionally, this discussion will examine the role of flow cytometric analysis and other markers of proliferative activity. Finally, we will consider some new genetic concepts as they relate to microsatellite instability and hereditary colorectal carcinoma will be considered.

Lymph Node Dissection

The single most important factor related to patient prognosis is the presence or absence of lymph node metastases. There is no doubt that searching for lymph nodes in a resection specimen is a tedious task. The lymph node yield per case is directly proportional to the dissector’s enthusiasm and skill. As a general rule, a “standard” resection specimen for carcinoma of the sigmoid colon or rectum should contain 10-25 lymph nodes, but we all have had cases where the dissector found far less. The question then is raised, should one routinely employ clearance techniques for lymph node detection? There are certainly advantages to these clearance techniques. One is likely to find all of the lymph nodes in a specimen and the lymph node yield will no longer depend upon the dissector’s ability and enthusiasm. However, the clearance process is time-consuming. It may delay the final pathology report up to two weeks in contrast to a 1-2 day turnaround time for routinely processed specimens (9, 10). Clearing is
expensive because large volumes of alcohol and xylene (or other clearing agent) are used and prolonged technologists’ time is required. The usual clearing agent, xylene is flammable and toxic. It can be absorbed through the skin and is currently considered a hazard in the histology laboratory.

Cawthorn et al (10), showed that clearance techniques increase the yield of lymph nodes per specimen when compared to routine dissection. However, the proportion of Dukes’ A, B, and C cases did not change (Table 15). The number of positive lymph nodes found was similar between cleared and non-cleared groups (Table 16). Clearance techniques are unnecessary for routine cases (8,11).

Additional controversy is added by consideration of non-traditional methods of lymph node examination such as immunohistochemistry for CEA, cytokeratins and epithelial membrane antigen or PCR testing looking for various tumor DNA or RNA. The biologic significance of these non-traditional methods lack validation (8, 12-15). Currently, the CAP recommends that all grossly identified lymph nodes be sectioned (without multiple levels) in a routine fashion. As a general rule, 12-15 negative lymph nodes usually correlate with true N0 status (16, 17).

**Histologic Grading**

Any pathologist will freely admit that grading in more art than science. Grading is subjective and prone to significant inter- and intraobserver variation. One multicenter trial noted 3% well-differentiated adenocarcinomas from one institution, while another hospital reported 97% well-differentiated cases (6). Marked heterogeneity exists within a given tumor. Some observers grade “on the average” while others assign a grade corresponding to the least differentiated area. Many grading systems are used for colorectal carcinoma (18-24). All employ slightly different criteria. Criteria are poorly defined in most systems. Some use three grades, and others four. Some exclude mucinous carcinoma altogether and others include it as grade IV or grade III. Criteria for mucinous carcinoma are almost never defined. Also notice that Dukes was involved in no less than three different grading systems; two with four grades, and one with three. Is it any wonder that grading is in such a state of disarray?

I continue to follow the guidelines of Dukes and Bussey (24) and use a three-grade system. Well-differentiated adenocarcinoma (grade I) shows tubular differentiation, the nuclear polarity is easily discerned, and nuclei are, in general, uniform in size. Moderately differentiated carcinomas exhibit a more complex and irregular tubular pattern, and the polarity of nuclei is lost or only barely discernible. Poorly differentiated adenocarcinoma (grade III) consists of highly irregular glands or may show an absence of glandular architecture. Nuclear polarity is lost. When variability exists within a given tumor, the grade is determined by the worst area no matter how small. Mucinous carcinoma and signet ring cell carcinoma are considered grade III (25). The relative distribution of cancer grades and corresponding survivorship is illustrated in Table 5.

Jass et al (6) attempted to establish a more scientific grading system based upon a Cox Regression Analysis Model. Although interesting, this study illustrates just how complicated things can get. This group reviewed 447 resection specimens and evaluated virtually every histologic feature. Table 15 illustrates features significantly related to survival after univariate analysis. The features that remained significant after Cox Multiple Regression Analysis were used to construct a scoring system with values weighted proportionally to the corresponding regression coefficient (Table 18). A histologic grading system was then designed that divided patients into four prognosis related groups (Table 19). These investigators then went further and added stage related parameters into the Cox Multiple Regression Model. After this analysis, only three factors emerged as significant: lymph node involvement, local spread (i.e., Dukes’ stage), and the amount of lymphocytic infiltration in the neoplasm (i.e., probably a
reflection of MSI status). Finally, they derived a prognosis-related scoring system based on stage and grade parameters (Table 20 and 21).

The Jass system is cumbersome and not widely used. The definition of local spread is not precisely outlined. The Jass system can only be used on resection specimens because two of the three independent variables (the pattern of growth and lymphocytic infiltration), cannot be adequately assessed in biopsy specimens. Of particular interest is the fact that after considering both staging and grading factors, the only significant histologic feature was the amount of lymphocytic infiltration. This apparent favorable prognostic effect occurs with marked lymphocytic infiltration only. This finding has been confirmed by others (26).

Probably the most important contribution of the Jass study (6) was that it provided the scientific verification for Dukes’ original observation that grade was subservient to stage in prognosis (24). It re-emphasized the need for careful specimen dissection to determine the amount of local spread and lymph node involvement.

The CAP has recently suggested that a 2-tiered grading system be adopted (8). The consensus panel believes that this would reduce interobserver variation while retaining the prognostic significance of grading.

**Histologic Type**

Many believe that mucinous carcinoma and signet ring cell carcinoma are associated with a prognosis significantly worse than nonmucinous adenocarcinoma. Unfortunately, the definitions of mucinous and signet ring cell carcinoma are not clear or are variable (8, 27-31). Work by Sasaki et al (30) and Umpleby et al (31), verifies that mucinous and signet ring cell carcinomas are indeed associated with a worse prognosis (Table 22). They tend to present at a high Dukes’ stage and are associated with extensive local spread.

Sasaki scrutinized a large cohort of mucinous, signet ring cell and nonmucinous carcinomas using a Cox Multiple Regression Model (30). According to this study, the only significant prognosis-related independent variables were the presence of lymph node metastases, extent of local spread (i.e., Dukes’ stage), infiltrative growth pattern, and lymphocytic infiltration. These latter two histologic features are not assessable in biopsy specimens. Both Sasaki and Umpleby groups concluded that mucinous carcinoma (greater than 75%-80% by volume) and signet cell carcinoma (greater than 50% cells signet ring) are more aggressive (30, 31). These histologies were not, however, significantly associated with poor prognosis if controlled for stage. In other words, their poor prognosis related to a more advanced stage at diagnosis (30).

**Flow Cytometry/DNA Content Variables/Proliferation Markers**

Flow cytometry for examination of DNA content in human tumors involves cells or isolated nuclei stained in suspension with a fluorescent dye that binds stoichiometrically with double-stranded DNA. These stained cells/nuclei are then passed one by one through the excitor light source (laser). The amount of fluorescence produced by the bound dye is detected by a photoelectric cell and the information is stored in a computer. With this technique, thousands of measurements can be made in seconds and displayed on a histogram. The position of peaks on the x-axis is proportional to the amount of DNA per cell, and height of peaks on the y-axis is proportional to the number of cells demonstrating that particular
DNA content. Using this method “diploid” cell populations can be distinguished from “non-diploid” (including aneuploid) cell populations (7).

Studies of paraffin-embedded and fresh colorectal carcinoma have demonstrated an inconsistent association between DNA aneuploidy and survival (26, 32-42). In at least one of these studies (26) stage was retained as a strong independent variable associated with prognosis after multiple regression analysis. Bauer et al (35), showed no independent association between DNA aneuploidy and prognosis in a large group of patients with colorectal cancer. In their experience, Dukes’ stage was the only independent variable associated with prognosis. When they looked at Dukes’ stage A and B cases alone, DNA aneuploidy was still not independently associated with adverse outcome. These authors showed that a high proportion of cells in the S phase was a significant independent variable associated with poor prognosis. The percent of cells in S phase, a measurement of proliferative activity, and had not been consistently measured in other studies.

DNA content analysis by flow cytometry is of no proved clinical value (8). The technology and methods lack standardization and in general the results between groups are not comparable. Most published studies have employed paraffin-embedded material. This is not optimal as DNA fragments and partial nuclei tend to stick together, leading to increased yields of “pseudo-aneuploid” histograms (43). The percent of cases showing aneuploidy peaks is lower when fresh intact cells are used. In terms of interpretation, control histograms are easy to read but in contrast tumor histograms are not clean and interpretations are subject to interobserver variation (not unlike histologic grading). Our study with 195 colorectal carcinomas prospectively studies by flow cytometric analysis showed no correlation between DNA aneuploidy and any standard staging or grading parameters (43) and no independent association with prognosis (44). The CAP believes that DNA analysis has been insufficiently studied for determination of prognostic value and that the data are insufficient to recommend a specific technological method (8).

We also analyzed a cohort of 122 patients with colorectal carcinoma utilizing an antibody that recognized Ki 67, a nuclear antigen expressed in all phases of the cell cycle except GO (45). We found no correlation between Ki 67 scores and stage, grade, or prognosis. Dukes’ stage, growth pattern, and lymphocytic infiltration were the only factors independently associated with prognosis. The CAP believes that there are insufficient data to recommend inclusion of proliferation indices for prognostic information (8).

**Early Non-Polypoid Colorectal Carcinoma**

Early invasive colorectal carcinoma (that is, invasive carcinoma limited to the submucosa) warrants special mention given the advent of modern endoscopic technology allowing gastroenterologists/surgeons an increased ability to resect carcinomas via the endoscope (e.g., endoscopic mucosal resection [EMR]). Given that lymph node status is the strongest prognostic factor in the evaluation of colorectal carcinoma, the question asked, particularly by colorectal surgeons, is whether or not a definitive surgical excision should be performed.

The issue is complicated by the low rate of lymph node metastases in pT1 colorectal carcinoma, estimated at 3-17% (46-49). We evaluated 132 pT1 colorectal carcinoma cases over an 11 year period (1987-1997) and found positive lymph nodes in 25 (19%) cases (48). 23 of the 25 positive lymph node cases (92%) had only 1 positive node. The high rate of metastases in our study could be due to the exclusion of malignant polyps, the technique of lymph node assessment (mean lymph node yield of 15) and the high incidence of micrometastic (<2mm) lymph node involvement (88% of cases). Follow-up in these cases...
did not reveal a single case of tumor recurrence, distal metastases or death from disease. One may argue that the surgical resection itself dramatically changed the natural course of the disease. Conversely, there is a case to support the good prognostic outcome of early colorectal carcinoma, thus justifying endoscopic removal if technically feasible and avoidance of surgical resection procedures associated with comorbidity and mortality.

This dilemma has prompted evaluation of histologic parameters and molecular markers that correlate with positive lymph node status in locally excised colorectal carcinoma with invasion limited to the submucosa. We evaluated multiple molecular markers using a multivariate logistic regression model including p53, MIB-1, BCL-2, BAX, cyclin-D1 and mismatch repair proteins MLH-1, MSH-2 and MSH-6 and found no statistically significant association between these markers and positive lymph node status (50). Histologic features correlated with positive lymph node status in submucosal colorectal carcinoma including angiolymphatic invasion, the presence of poor tumor differentiation, tumor budding and SM3 invasion (invasion of the deepest 1/3 of the submucosa).

FAMILIAL COLORECTAL CARCINOMA AND NEW GENETIC CONCEPTS

Introduction

Approximately 80% of colorectal carcinomas appear sporadic whereas 20% potentially have a genetic cause (51). This latter group includes the 3% of cases thought to be related to hereditary non-polyposis colon cancer syndrome (HNPCC) and the 1% associated with familial adenomatous polyposis (FAP). Colorectal carcinoma can also be viewed another way. About 85% of colorectal cancers are thought to originate through the chromosomal instability pathway. These tumors are typically DNA aneuploid and demonstrate abnormalities of chromosomes 5, 17, 18. Familial adenomatous polyposis colon cancers arise via this pathway. Approximately 15% of colorectal carcinoma appears to arise in the so-called “mutator phenotype”. These cancers tend to be DNA diploid and are associated with microsatellite instability. The HNPCC cancers are associated with the “mutator phenotype.”

Microsatellite Instability

DNA integrity is essential for normal cell function. DNA insults can occur due to the direct effects of chemical carcinogens or radiation and are usually corrected through the excision repair system. DNA replication errors are of 2 types 1) simple mispairing of nucleotides, the most common DNA error type, and 2) “slipping” errors, where genes may contain too many or too few copies of repeat short DNA nucleotides known as “microsatellite” DNA sequences. Normally, these errors are recognized, the cell cycle arrested and the mismatched segment corrected. For those errors not immediately corrected by DNA polymerase, the mismatch repair (MMR) system acts as a back-up system allowing additional proof reading of DNA. Failure to repair mismatches will allow the error (mutation) to persist and to become a template for subsequent DNA replication (52). The known mismatch repair genes and their relative frequency in HNPCC are presented in Table 23.

Microsatellite instability is a useful epiphenomenon found in colorectal tumor DNA but not in adjacent non-neoplastic mucosa. It is a marker that indicates that extensive mutation exists in the non-encoding repetitive DNA sequences that are particularly prone to replication error (microsatellites). Microsatellite instability is detected in 15% of colorectal cancers and is present in over 90% of the cancers found in patients with HNPCC. The majority of microsatellite instability is linked to somatic inactivation of
mismatch repair genes through hypermethylation but it also can be detected in persons with germline mismatch repair gene mutations (HNPCC) (52).

HNPCC patients have a germline mutation of mismatch repair genes which confers an increased lifetime risk for colorectal and other cancers (51). These cancers develop at significantly younger ages. Other HNPCC related tumors include cancers of the endometrium, ovary, stomach, urinary tract, kidney and central nervous system.

Identification of patients and families can sometimes be done by taking a careful patient and family medical history, can be suggested from the pathologic findings of excised tumors, and can be determined by special testing. Pathologic features that suggest microsatellite instability/HNPCC include right-sided location, synchronous or metachronous cancers, large bulky polypoid tumors with circumscribed pushing borders, tumors showing prominent lymphoid infiltrate, cancers of poor differentiation (medullary carcinoma) or mucinous and signet ring histology (8, 51).

The diagnosis of HNPCC is evolving. Originally, the Amsterdam criteria were used to clinically identify HNPCC patients (53). The original Amsterdam criteria include; a) three or more relatives with a colorectal cancer with at least one a first-degree relative; b) colorectal carcinoma in two generations; and c) one or more colorectal carcinomas occurring in a person less than 50 years of age (Table 24). In order to increase the sensitivity, the Amsterdam criteria were later modified to include; a) three or more relatives with any HNPCC related carcinoma; b) colorectal carcinoma in two generations; and c) and one or more HNPCC related carcinoma in a person younger than 50 years of age (54). Problems with the Amsterdam criteria are many. Small families or patients in whom the family history is unknown or incomplete limit the utility of these criteria. Physician history taking is often not thorough. More importantly, up to 33% of Amsterdam negative people have germline mutations of the mismatch repair gene and only 60% of Amsterdam positive people have detectable mutations (54,58,59). Importantly, clinicians must still consider HNPCC when a suspicious family history does not fulfill the Amsterdam criteria.

Special testing (MSI testing) now augments the clinical criteria. The role of MSI analysis had led to the development of the Bethesda criteria as a guideline for testing colorectal tumors for microsatellite instability (55, 56). The original Bethesda criteria included a) persons meeting Amsterdam criteria, b) persons with two or more HNPCC-related cancers, c) persons with colorectal cancer and a first degree relative with an HNPCC cancer and/or colorectal adenoma (cancer less than 45 years and adenoma less than 40 years), d) colorectal or endometrial cancer in a patient less than 45 years of age, e) colorectal cancer with MSI high pathology, f) colorectal adenoma in a patient less than 40 years of age (Table 24). These guidelines were only recently modified (56a) and just one of the following criteria needs to be met: colorectal cancer before age 50, synchronous or metachronous colorectal or other HNPCC-related tumor regardless of age, colorectal cancer with MSI-high pathology in a patient less than 60, person with colorectal cancer and a first-degree relative with colorectal or other HNPCC-related tumor (cancer less than 50; adenoma less than 40), colorectal cancer with two or more relatives with colorectal or other HNPCC-related tumor regardless of age.

The American Gastroenterological Association position is that testing should be performed on families meeting Amsterdam criteria, on any affected person meeting the modified Bethesda criteria and any first-degree relative of those with known mutations of mismatch repair genes (51). They suggest that following pre-test genetic counseling and written form consent, immunohistochemistry for MSH2/MLH1 on tumor tissue and MSI testing be performed. In the MSI high cases, immunohistochemistry can direct gene sequencing. Almost all MSI-H cancers can be identified if the antibody panel includes PMS2 and

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MSH6 as well as MLH1 and MSH2 (57). In fact, MLH1 and MSH2 account for almost 90% of the observed mutations in HNPCC (58). In MSI-L or MSI-S tumors, no further testing is indicated. The clinical significance of molecular testing is that affected individuals and at risk persons are identified and can be treated with correct surgery. Furthermore, clinicians can institute proper screening such as colonoscopy at an earlier age, (age 25 or 5 years younger than the youngest cancer in the family), periodic endometrial sampling, ultrasound, CA125 serum testing and urine analysis for urinary tract carcinoma.

MSI in sporadic colorectal carcinoma is a subject of considerable contemporary interest. Much like their HNPCC counterparts there is also a predilection for the right colon, mucinous histology and a prominent lymphoid infiltrate (60). There are strong arguments for routine testing for MSI in all resected colorectal carcinoma, the most important of which is the lower mortality rate independent of tumor stage. Specifically, a 3-year recurrence free survival of 90% in MSI-H tumors versus 32% in MSI-L/MSI-S tumors (61,62). The molecular basis for the improved prognosis is unclear, however, it is postulated that mutations accumulate in genes that are necessary for tumor survival thus undergoing a “self-destruct” sequence (59). MSI-H is also associated with an increased rate of metachronous tumors with subsequent clinical implications for cancer surveillance and follow-up.

The relationship between MSI-H tumors and chemotherapy response is debated. In a study of stage 3 colorectal carcinomas, Elselah et al. found a survival benefit only in patients with MSI-H tumors receiving adjuvant fluorouracil chemotherapy (63). Suggesting that MSI status could act as a favorable prognostic marker for those undergoing adjuvant fluorouracil chemotherapy. In contrast, a study of MSI tumors in a recent randomized controlled trial assessing adjuvant fluorouracil chemotherapy found that patients with MSI-H tumors not receiving adjuvant fluorouracil chemotherapy had a better 5-year survival. However, among those receiving adjuvant fluorouracil chemotherapy survival was only improved in the MSI-L/MSI-S group with no benefit observed for the MSI-H group after adjusting for tumor grade and stage (64).

REFERENCES


49. Goldstein NS, Hart J. Histologic features associated with lymph node metastasis in T1 and superficial T2 rectal adenocarcinomas in abdominoperineal resection specimens. Identifying a subset of patients for whom treatment with adjuvant therapy or completion abdominoperineal resection should be considered after local excision. Am J Clin Pathol. 111:51-58, 1999.


### TABLE 1 - SURVIVAL RELATED TO CLINICOPATHOLOGIC STAGE (1)

<table>
<thead>
<tr>
<th>STAGE</th>
<th>NUMBER OF CASES</th>
<th>CORRECTED 5 YEAR SURVIVAL</th>
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<tbody>
<tr>
<td>A</td>
<td>103</td>
<td>98.9</td>
</tr>
<tr>
<td>B</td>
<td>212</td>
<td>84.9</td>
</tr>
<tr>
<td>C</td>
<td>156</td>
<td>67.3</td>
</tr>
<tr>
<td>D</td>
<td>205</td>
<td>14.3</td>
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### TABLE 2 - SURVIVAL RELATED TO EXTENT OF LOCAL SPREAD FOR DUKE’S B CASES (2)

<table>
<thead>
<tr>
<th>LOCAL SPREAD</th>
<th>5 YEAR SURVIVAL</th>
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<tr>
<td>Slight</td>
<td>90%</td>
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<tr>
<td>Moderate</td>
<td>80 %</td>
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<tr>
<td>Extensive</td>
<td>57%</td>
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### TABLE 3 - SURVIVAL RELATED TO LYMPH NODE INVOLVEMENT (2)

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<th>STAGE</th>
<th>5 YEAR SURVIVAL</th>
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<tbody>
<tr>
<td>Dukes’ A &amp; B cases</td>
<td>80%</td>
</tr>
<tr>
<td>Dukes’ C cases</td>
<td>30%</td>
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### TABLE 4 - SURVIVAL RELATED TO NUMBERS OF INVOLVED LYMPH NODES (2)

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<tr>
<th>NUMBER OF NODES POSITIVE</th>
<th>5 YEAR SURVIVAL</th>
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<tr>
<td>0</td>
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</tr>
<tr>
<td>1</td>
<td>60%</td>
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<td>2 – 5</td>
<td>35%</td>
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<td>6 or more</td>
<td>20%</td>
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### TABLE 5 - SURVIVAL RELATED TO HISTOLOGIC GRADE (2)

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<th>GRADE</th>
<th>PERCENT TOTAL</th>
<th>CORRECTED 5 YEAR SURVIVAL</th>
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<tr>
<td>1</td>
<td>20%</td>
<td>86%</td>
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<tr>
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<td>61%</td>
<td>60%</td>
</tr>
<tr>
<td>3</td>
<td>19%</td>
<td>20%</td>
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### TABLE 6 - SURVIVAL RELATED TO VENOUS INVASION: ALL CASES (3)

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<tr>
<th>VENOUS INVASION</th>
<th>CORRECTED 5 YEAR SURVIVAL</th>
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<tr>
<td>Not demonstrated</td>
<td>73%</td>
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<td>Present:</td>
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<td>Intramural</td>
<td>66%</td>
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<tr>
<td>Extramural</td>
<td>33%</td>
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### TABLE 7 - SURVIVAL RELATED TO VENOUS INVASION FOR DUKES’ B CASES (3)

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<th>VENOUS INVASION</th>
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<tr>
<td>Not demonstrated</td>
<td>85.6%</td>
</tr>
<tr>
<td>Present</td>
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<tr>
<td>Intramural</td>
<td>85.3%</td>
</tr>
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<td>Extramural</td>
<td>64.0%</td>
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### TABLE 8 - SURVIVAL RELATED TO VENOUS INVASION FOR DUKES’ CASES (3)

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<td>Not demonstrated</td>
<td>45.7%</td>
</tr>
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</tr>
<tr>
<td>Intramural</td>
<td>40.5%</td>
</tr>
<tr>
<td>Extramural</td>
<td>17.3%</td>
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TABLE 9 - PREVALENCE OF LIVER METASTASES RELATED TO VENOUS INVASION (3)

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<th>VENOUS INVASION</th>
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<tr>
<td>Not demonstrated</td>
<td>14.2%</td>
</tr>
<tr>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>Intramural</td>
<td>23.4%</td>
</tr>
<tr>
<td>Extramural</td>
<td>40.2%</td>
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TABLE 10 - SURVIVAL RELATED TO FREE MESOTHELIAL SURFACE INVASION FOR DUKE’S B CASES (4)

<table>
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<th>FEATURE</th>
<th>NUMBER OF CASES</th>
<th>CORRECTED 5 YEAR SURVIVAL</th>
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<tr>
<td>No surface invasion</td>
<td>380</td>
<td>77%</td>
</tr>
<tr>
<td>Surface invasion present</td>
<td>27</td>
<td>54%</td>
</tr>
</tbody>
</table>

TABLE 11 - PROGNOSTIC FACTORS THAT APPEAR TO BE ASSOCIATED WITH PROGNOSIS BUT REQUIRE ADDITIONAL STUDY (8)

- Histologic type
- Histologic features associated with microsatellite instability
  - Lymphoid response
  - Medulary carcinoma
  - Mucinous carcinoma
- MSI-High
- LOH of 18q (DCC gene allelic loss)
- Tumor border configuration (expanding vs. infiltrative)

TABLE 12 - FACTORS NOT YET SUFFICIENTLY STUDIED TO DETERMINE PROGNOSTIC VALUE (8)

- DNA content
- Perineural invasion
- Microvessel density
- Peritumoral fibrosis
- Peritumoral inflammatory response
- Focal neuroendocrine differentiation
- Nucleolar organizing region
- Proliferative indices

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**TABLE 13 - MOLECULAR MARKERS NOT YET SUFFICIENTLY STUDIED TO DETERMINE PROGNOSTIC VALUE (8)**

- Tumor suppressor genes
  - LOH of 1p, 8p, 1p, 5q
- Oncogenes
  - K-ras, c-myc
- Apoptosis related genes
  - bcl-2, BAX
- DNA synthesis-related genes
  - thymidylate synthase/phosphatase
- Growth factor genes
  - EGF-R, TGF-α, TGF-β, her2/neu
- Cyclin-dependent kinase inhibitor genes
  - p27, p21
- Angiogenesis-related genes
  - VEGF
- Adhesion molecule/glycoprotein genes
  - CD44, E-cadherin, sialo-Tn antigen
- Matrix metalloprotein and inhibitor genes
  - urokinase-type plasminogen activator
- Metastasis suppressor gene
  - nm23-H1

**TABLE 14 - CELL PROTEINS AND CARBOHYDRATES NOT SUFFICIENTLY STUDIED TO DETERMINE PROGNOSTIC VAULE (8)**

- Class I HLA molecules
- Class II HLA molecules
- CA 19-9
- CA 72-4
- Sialyl Le
- Sialosyl-Tn
- Urokinase-type plasminogen activator
- Plasminogen activator inhibitor 2
- Glycoprotein 72
- Laminin
- MUC-1 mucin
- E-cadherin
- α-Catenin
- Integrins
- Type IV collagen
- Gelatinase B (metalloproteinase-9)
- Tenascin
- Autocrine mobility factor receptor gp78)
- Phospholipase C
- Secretory component of immunoglobulin A
- Metallothionein
- EGF-R
- Gastrin receptor
- Somatostatin receptors
- Cathepsin B, L, and D (cysteine/aspartyl proteases)
- Ferritin
- CD44
- Vitamin D receptor protein
- Cytokeratin 20
- P-glycoprotein (multidrug resistance gene product)
### TABLE 15 - DISTRIBUTION OF DUKES’ STAGE FOR RECTAL CANCER: COMPARISON BETWEEN STANDARD LYMPH NODE DISSECTION AND LYMPH NODE CLEARANCE TECHNIQUE (10)

<table>
<thead>
<tr>
<th>STAGE</th>
<th>LYMPH NODE CLEARED</th>
<th>LYMPH NODES NOT CLEARED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dukes’ A</td>
<td>18%</td>
<td>16%</td>
</tr>
<tr>
<td>Dukes’ B</td>
<td>29%</td>
<td>34%</td>
</tr>
<tr>
<td>Dukes’ C</td>
<td>53%</td>
<td>50%</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

### TABLE 16 - RELATIVE DISTRIBUTION OF THE NUMBER OF POSITIVE LYMPH NODES FOR DUKES’ C CASES: A COMPARISON OF STANDARD LYMPH NODE DISSECTION TO LYMPH NODE CLEARANCE TECHNIQUE (10)

<table>
<thead>
<tr>
<th>NODES POSITIVE</th>
<th>LYMPH NODES CLEARED</th>
<th>LYMPH NODES NOT CLEARED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26%</td>
<td>24%</td>
</tr>
<tr>
<td>2</td>
<td>15%</td>
<td>16%</td>
</tr>
<tr>
<td>3</td>
<td>7%</td>
<td>4%</td>
</tr>
<tr>
<td>4 or more</td>
<td>52%</td>
<td>56%</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

### TABLE 17 - HISTOLOGIC FEATURES SIGNIFICANTLY RELATED TO SURVIVAL AFTER UNIVARIATE ANALYSIS (6)

1. TUMOR TYPE – papillary > tubular > mucinous/signet ring
2. DIFFERENTIATION – well > moderate > poor
3. NUCLEAR POLARITY – easily discerned > irregular tubules > no tubules
4. TUBULE CONFIGURATION – regular > irregular > none
5. GROWTH PATTERN – expanding > infiltrating
6. FIBROSIS – slight > moderate > extensive
7. LYMPHOCYTIC INFILTRATION – marked > moderate > little

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### TABLE 18 - SCORING SYSTEM FOR MODEL BASED ON GRADE RELATED PARAMETERS (6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubule configuration</td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>0</td>
</tr>
<tr>
<td>Irregular</td>
<td>2</td>
</tr>
<tr>
<td>None</td>
<td>4</td>
</tr>
<tr>
<td>Pattern of growth:</td>
<td></td>
</tr>
<tr>
<td>Expanding</td>
<td>0</td>
</tr>
<tr>
<td>Infiltrating</td>
<td>2</td>
</tr>
<tr>
<td>Lymphocytic infiltration</td>
<td></td>
</tr>
<tr>
<td>Marked</td>
<td>0</td>
</tr>
<tr>
<td>Moderate</td>
<td>3</td>
</tr>
<tr>
<td>Little</td>
<td>6</td>
</tr>
</tbody>
</table>

### TABLE 19 - SURVIVAL BASED ON PATHOLOGICAL GRADING PARAMETERS (6)

<table>
<thead>
<tr>
<th>GRADE</th>
<th>SCORE</th>
<th>NUMBER OF CASES</th>
<th>CORRECTED 5 YEAR SURVIVAL</th>
<th>CORRECTED 10 YEAR SURVIVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>102</td>
<td>93%</td>
<td>87%</td>
</tr>
<tr>
<td>II</td>
<td>1 – 4</td>
<td>134</td>
<td>70%</td>
<td>66%</td>
</tr>
<tr>
<td>III</td>
<td>5 – 8</td>
<td>119</td>
<td>52%</td>
<td>46%</td>
</tr>
<tr>
<td>IV</td>
<td>9 – 12</td>
<td>92</td>
<td>20%</td>
<td>14%</td>
</tr>
</tbody>
</table>
### TABLE 20 - SCORING SYSTEM FROM MODEL BASED ON GRADE AND STAGE RELATED PARAMETERS (6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytic infiltration</td>
<td></td>
</tr>
<tr>
<td>Marked</td>
<td>0</td>
</tr>
<tr>
<td>Moderate</td>
<td>3</td>
</tr>
<tr>
<td>Little</td>
<td>6</td>
</tr>
<tr>
<td>Node involvement</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 – 4</td>
<td>4</td>
</tr>
<tr>
<td>5 or more</td>
<td>8</td>
</tr>
<tr>
<td>Local spread:</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Slight to moderate</td>
<td>3</td>
</tr>
<tr>
<td>Extensive</td>
<td>6</td>
</tr>
</tbody>
</table>

### TABLE 21 - SURVIVAL BASED ON GRADE AND STAGE RELATED PARAMETERS (6)

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>SCORE</th>
<th>NUMBER OF PATIENTS</th>
<th>CORRECTED 5 YEAR SURVIVAL</th>
<th>CORRECTED 10 YEAR SURVIVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>32</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>II</td>
<td>1 – 6</td>
<td>135</td>
<td>88</td>
<td>86</td>
</tr>
<tr>
<td>III</td>
<td>7 – 11</td>
<td>110</td>
<td>72</td>
<td>65</td>
</tr>
<tr>
<td>IV</td>
<td>12 – 16</td>
<td>115</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td>V</td>
<td>17 – 20</td>
<td>56</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

### TABLE 22 - RECTAL CARCINOMA: SURVIVAL RELATED TO HISTOLOGIC SUBTYPES (30)

<table>
<thead>
<tr>
<th>- TYPE</th>
<th>NUMBER OF CASES</th>
<th>5 YEAR SURVIVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonmucinous</td>
<td>413</td>
<td>62%</td>
</tr>
<tr>
<td>Mucinous (less than 75%)</td>
<td>175</td>
<td>60%</td>
</tr>
<tr>
<td>Mucinous (greater than 75%)</td>
<td>141</td>
<td>53%</td>
</tr>
<tr>
<td>Signet ring</td>
<td>45</td>
<td>13%</td>
</tr>
</tbody>
</table>

### TABLE 23 - MISMATCH REPAIR GENE AND FREQUENCES IN HNPCC

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<table>
<thead>
<tr>
<th>GENE</th>
<th>FREQUENCY</th>
<th>LOCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMLH1</td>
<td>49%</td>
<td>3p21</td>
</tr>
<tr>
<td>hMSH2</td>
<td>45%</td>
<td>2p15</td>
</tr>
<tr>
<td>hPMS2</td>
<td>4%</td>
<td>7p22</td>
</tr>
<tr>
<td>hPMS1</td>
<td>1%</td>
<td>2p32</td>
</tr>
<tr>
<td>hMSH6</td>
<td>1%</td>
<td>2p15</td>
</tr>
<tr>
<td>hMSH3</td>
<td>0%</td>
<td>5q11-13</td>
</tr>
</tbody>
</table>

**TABLE 24 - CLINICAL CRITERIA AND GUIDELINES FOR HEREDITARY NON POLYPOSIS COLORECTAL CARCINOMA**

I. Amsterdam criteria (All criteria must be met):
   At least 3 relatives with colorectal cancer plus all of the following:
   1. One member diagnosed with colorectal cancer before age 50 years
   2. Two affected generations
   3. Three affected relatives, one of them a first-degree relative of the other two
   4. FAP should be excluded
   5. Tumors should be verified by pathologic examination

II. Amsterdam criteria II (All criteria must be met)
   At least 3 relatives with an HNPCC-associated cancer (colorectal cancer or cancer of the endometrium, small bowel, ureter, or renal pelvis) plus all of the following:
   1. One should be a first-degree relative of the other two
   2. At least 2 successive generations should be affected
   3. At least one should be diagnosed before age 50 years
   4. FAP excluded in any case of colorectal cancer
   5. Tumors should be verified by pathologic examination

III. Bethesda guidelines (for identification of patients with colorectal tumors who should undergo testing for microsatellite instability)
   1. Individuals with cancer in families that meet the Amsterdam criteria
   2. Individuals with 2 HNPPC-related cancers, including synchronous and metachronous colorectal cancers or associated extracolonic cancers (Note: endometrial, ovarian, gastric, hepatobiliary, or small bowel cancer or transitional cell carcinoma of the renal pelvis or ureter)
   3. Individuals with colorectal cancer and a first-degree relative with colorectal cancer and/or HNPPC-related extracolonic cancer and/or a colorectal adenoma; one of the cancers diagnosed at age younger than 45 years, and the adenoma diagnosed at age younger than 40 years
   4. Individuals with colorectal cancer or endometrial cancer diagnosed at age younger than 45 years
   5. Individuals with right-sided colorectal cancer with an undifferentiated pattern (solid/cribriform) on histopathology diagnosed at age younger than 45 years (Note: solid/cribriform defined as poorly differentiated or undifferentiated carcinoma composed of irregular, solid sheets of large eosinophilic cells and containing small gland-like spaces)
   6. Individuals with signet-ring cell type colorectal cancer diagnosed at age younger than 45 years (Note: composed of > 50% signet-ring cells. A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: Meeting highlights and Bethesda guidelines**)
   7. Individuals with adenomas diagnosed at age younger than 40 years

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* Differences between Amsterdam and Amsterdam II underlined

THE COLORECTAL SERRATED POLYP FAMILY

Colonic Hyperplastic Polyps and Hyperplastic Polyposis

Hyperplastic polyp is the most common benign polyp of the large intestine (1). These polyps are usually small (less than 5 mm), sessile and often about the same color as the surrounding colonic mucosa. Histologically, absorptive and goblet line crypts that are elongate and dilated. Since there are more epithelial cells per unit area than normal, the cells tend to pseudostratify, imparting a serrated or micropapillary appearance. Characteristically, the basement membrane under the surface epithelium is thickened and hyalinized. Regenerative epithelial changes are mitoses can be quite prominent at the crypt bases. This regenerative area can occasionally cause diagnostic confusion with carcinoma, especially in a variant referred to as inverted hyperplastic polyp (2, 3). In the inverted variety, the regenerative epithelium of the crypt base of the hyperplastic polyp is misplaced into or beneath the muscularis mucosae. This variant which is probably best classified as a sessile serrated polyp (see below) is easily recognized if one is cognizant of its existence and also notes the overall architecture and cytology of a hyperplastic polyp. The entity is distinguished from invasive adenocarcinoma by the lack of infiltration and tumor desmoplasia.

The differential diagnosis between hyperplastic polyp and tubular adenoma can also be difficult, especially in a diminutive polyp that has been treated by hot biopsy (so-called “Thermal Polyp”). The features that I find useful in the differential are found in the table.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Hyperplastic Polyp</th>
<th>Tubular Adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regenerative Zone</td>
<td>Basal</td>
<td>Surface</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Usually No</td>
<td>Yes</td>
</tr>
<tr>
<td>Hyalinized basement membrane</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

In a “tight call”, as long as an adenoma diagnosis is not going to result in a surgical resection (e.g., right colonic adenoma incompletely excised), I tend to error on the side of the adenoma since the patient will receive more frequent and careful surveillance. Mixtures of hyperplastic polyp and adenoma do exist (4, 5). Many consider illustrated as “serrated adenoma”as a variant of villous adenoma.
Hyperplastic Polyposis

Rare examples of patients with colons carpeted by hyperplastic polyps (so-called hyperplastic polyposis) have been described. Hyperplastic polyposis is probably a heterogeneous disorder with some forms associated with high microsatellite instability (MSI-H) cancers in which there is methylation with loss of expression of hMLH1 (6, 7). Therefore, hyperplastic polyposis may be a marker for the so-called “methylator phenotype”. These patients may be prone to colorectal carcinoma. Some cases have shown evidence of inheritance presumably caused by a genetic predisposition to methylation. The type and order of methylated genes varies and may account for the microsatellite stable (MSS), low microsatellite instability (MSI-L) and MSI-H cancers described. When several cancers in hyperplastic polyposis families are MSI-H, the distinction from hereditary non-polyposis colon cancer syndrome can be difficult. Features that favor hyperplastic polyposis include; background serrated adenomas, presence of some MSS or MSI-L cancers in the kindred, older age at onset of cancer, limited numbers of affected family members, methylation of hMLH1 and failure to detect germline mutation of mismatch repair genes.

Unusual “Hyperplastic” Polyps and Possible Relationship to Colorectal Carcinoma (7-12)

Several lines of reasoning link “hyperplastic polyps” with colorectal carcinoma. Investigators report individual cases and small series of carcinoma complicating hyperplastic polyps. The association between colorectal cancer and hyperplastic polyposis has already been noted above. There is a high rate of co-existing hyperplastic polyps and not adenomas in patients with MSI-H carcinoma. Methylation induced inactivation of mismatch repair genes occurs in both hyperplastic polyps and carcinoma. Recently, a large series of MSI-H colorectal carcinoma predated by biopsied proved “hyperplastic polyps” at the same site has been reported.

These “hyperplastic polyps” associated with carcinoma have often been unusually large, right sided and have been reported under a number of synonyms including giant hyperplastic polyp, sessile serrated adenoma, sessile serrated polyp, inverted hyperplastic polyp, and polyp with epithelial serrated proliferation.

It is becoming clear that there may be several entities that have been called “hyperplastic polyps” in the past. This serrated polyp family includes conventional hyperplastic polyp, mixed hyperplastic polyp/adenoma, serrated adenoma (epithelial dysplasia defined) and hyperplastic-like polyps with unusual features which have been most frequently referred to as sessile serrated polyps. Sessile serrated polyps as the name implies are sessile, large (frequently 1 cm more), right sided, and show poor endoscopic circumscription. A number of cytological and architectural abnormalities have been reported in the sessile serrated polyp, especially those that have been associated with carcinoma. The abnormal proliferation/dysmaturation features include persisting nuclear atypia with large nuclei and nucleoli high in the crypts, high mitoses figures and irregular distribution of dystrophic goblet cell. Architectural abnormalities include basal crypt dilatation, horizontally oriented crypts, inverted crypts, prominent serration and the lack of the surface basement membrane thickening typical of convention hyperplastic polyps.

Once recognized, the sessile serrated polyp creates a patient management dilemma. Calling them serrated adenomas may not be an appropriate default diagnosis because it is unknown whether colonic resection would be required for incomplete excision. (This is typically done for incompletely excised adenomas). Furthermore, endoscopic follow-up for serrated adenoma would typically be at a three or five year interval. In the cohort of 91 patients with sessile serrated polyps proceeding MSI-H carcinomas, 19 predated the carcinomas by less than three years (Goldstein NS, et al. American Journal of Clinical
Pathology 119:778, 2003). Perhaps a shorter surveillance interval (e.g., 1-2 years) is better for these types of polyps that are incompletely excised or associated with additional similar endoscopically appearing polyps that have remained unsampled.

REFERENCES


Epidermal growth factor receptor (EGFR) Immunohistochemistry in metastatic colorectal cancer

Background

Cetuximab (Erbitux) is a monoclonal antibody specifically designed to bind EGFR-1, blocking binding of certain growth factors as well as cell signaling to promote tumor cell growth, survival and progression. EGFR-1 is a protein expressed in a variety of cancers including colorectal, breast, pancreatic, bladder, prostate, lung, kidney and head and neck tumors.

In February 2004 Erbitux received FDA approval to treat EGFR-1 expressing metastatic colon cancers which are refractory or intolerant to irinotecan. Eligibility to receive Erbitux treatment is based on the presence of EGFR-1 expression by immunohistochemical evaluation using the DakoCytomation EGFR pharmDx test kit.

The EGFR-1 Pathway

The epidermal growth factor receptor 1 (HER1, c-ErbB-1) is a 170kDa transmembrane glycoprotein encoded by the human HER1 gene, being a member of the tyrosine kinase growth factor receptor protein family. EGFR is expressed in normal epithelium including the skin, follicular and cervical epithelium.
Ligand binding of EGFR-1 results in dimerization of the receptor. Subsequent autophosphorylation of tyrosine kinase initiates intracellular signaling, resulting in a number of effects which increase tumor cell growth. 14-18

**Erbitux therapy and inhibition of EGFR expression**

Erbitux (Cetuximab) is a chimeric (mouse/human) monoclonal antibody specifically designed to bind the extra-cellular domain of EGFR-1 on both normal and tumor cells. Erbitux competitively inhibits the binding of epidermal growth factor and other ligands (eg. TGF-α) by preferentially binding to EGFR-1. 14-18 Subsequently, Erbitux inhibits ligand-induced tyrosine kinase autophosphorylation with multiple effects including inhibition of cell growth, induction of apoptosis, decreased production of vascular endothelial growth factor (VEGF) and decreased production of matrix metalloproteinase (MMP). 14-18

In vitro assays and in vivo animal studies have demonstrated that Erbitux inhibits growth and survival of tumor cells that express EGFR-1. 1-6 The addition of Erbitux to irinotecan or irinotecan plus 5-fluorouracil in animal studies resulted in increased antitumor effects compared to these chemotherapy agents alone. 1-6

**Erbitux Clinical Trials**

Erbitux is FDA approved to be used in combination with irinotecan in patients in which the colorectal carcinoma (CRC) expresses EGFR-1. 1 Three CRC Erbitux clinical trials have been performed using EGFR immunohistochemical expression as a criterion for study eligibility. 1 In a pivotal randomized control trial (EMD 62202-007) 329 patients with metastatic colorectal carcinoma with EGFR-1 expression were randomized to receive varying doses of Erbitux alone or in combination with irinotecan. 1 EGFR-1 expression in all cases was determined by immunohistochemistry (DakoCytomation EGFR pharmDx test kit). In this study all patients enrolled were required to have immunohistochemical evidence of positive EGFR-1 expression. Study subpopulations included irinotecan refractory patients as well as irinotecan refractory and oxaliplatin failed treatment patient groups. EGFR-1 expression is known to be present in up to 77% of colorectal carcinomas. 8,10-11 In this trial 82.1% of tumor specimens were EGFR-1 positive, and objective response rates of 22.9% and 10.8%, respectively, were seen in the Erbitux with irinotecan (n=218) and the Erbitux as a single agent (n=111) patient groups. The time to progression in all patients was a median of 4.1 months on the Erbitux with irinotecan group and 1.5 months in the Erbitux as a single agent group. Thus demonstrating a synergistic response of Erbitux with irinotecan.

**EGFR-1 Immunohistochemistry**

An important finding of the randomized pivotal trial with respect to EGFR-1 immunohistochemical expression was the lack of correlation of either the percentage of positive cells or the intensity of EGFR expression with response rate. 1 Consequently, the threshold for a positive immunohistochemical result is set at 1% of tumor cells or greater regardless of the degree of staining intensity. The DakoCytomation EGFR pharmDx test kit includes all necessary reagents to perform the test including pre-diluted antibody, labeled polymer, DAB, control slides (cell line) and proteinase K. The intended staining pattern is cytoplasmic membrane staining best demonstrated in the most poorly differentiated component of the tumor, usually located at the leading edge. The EGFR-1 positive cell line slide included with the kit should demonstrate at least 2+ cytoplasmic membrane staining. In addition, EGFR-1 expression is seen in the perineurium surrounding small nerves in the colon, which acts as a useful internal control.
Recommended positive control tissue is cervical squamous epithelium which demonstrates cytoplasmic membrane staining which is strongest in the basal aspect of the squamous epithelium. Very little data exists regarding correlation between EGFR-1 expression in primary and metastatic tumors including metastatic carcinoma within lymph nodes, however, in practice negative expression in a primary tumor mandates additional evaluation of metastatic tumor in lymph nodes, liver or lung. EGFR-1 results should be reported as positive or negative. The presence of abundant intracytoplasmic immunoreactivity is generally associated with the presence of some cytoplasmic membrane staining which can be found in most cases on close inspection.

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homology to EGF receptor shares chromosomal location with neu oncogene. Science 1985; 230:1132


DYSPLASIA AND CANCER IN INFLAMMATORY BOWEL DISEASE

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AmeriPath, Inc.
Oakwood Village, Ohio

Ulcerative Colitis, Dysplasia, and Carcinoma

Patients with long-standing ulcerative colitis (UC) are at increased risk for colorectal adenocarcinoma (1). Those at greatest risk are patients with extensive colitis (2,3) who have been afflicted for more than eight to ten years. Early age at onset of colitis and sclerosing cholangitis may also be associated with increased risk (2,4).

Colitis-associated carcinoma makes up only a small proportion of total colorectal cancer, but these malignancies are particularly bothersome to the medical community. They frequently occur in young patients and often develop insidiously even while the patient is under active medical care. Many times the carcinomas are not clinically apparent until distant spread has occurred (5,6). Colitis-associated carcinomas are often flat and infiltrative and therefore difficult to visualize with standard radiologic techniques. Since the symptoms associated with carcinoma mimic those of active colitis, diagnosis is often delayed (1,7).

The estimated risk of carcinoma complicating ulcerative colitis varies considerably and has been reported as low as 1.4% at 18 years (8). This study, however, is limited by very short median follow-up (only 6.7 years). Others have reported cumulative risks as high as 34% at 25 years (9) and up to 60% for patients with extensive colitis for more than 30 years (3,10-12). Serious methodologic problems surround these current assessments of cancer risk in UC. Those that lead to overestimation of the cancer risk include the use of referral center populations, inclusion of patients already known to have cancer, and most importantly, projections of cancer risk using small numbers of patients. Although the exact magnitude of the cancer risk in UC is unknown, it is certainly higher than in patients who do not have colitis. Epidemiologic data suggest that patients with ulcerative colitis have six to eight times the risk of developing carcinoma when compared to the general population and looking at the subgroup of colitis with extensive colitis, the risk is fifteen- to nineteen-fold (2,13). This increased risk poses a considerable management dilemma for physicians caring for patients not sick enough to require colectomy.

Several management options are available. First, the physician can ignore the risk. Many prefer this approach when dealing with an older patient, or a patient who is not a surgical candidate, especially if one believes that the actual cancer risk is low (10). A second management approach is “prophylactic
"prophylactic colectomy" in patients with colitis for longer than eight to 10 years (10-12). This may be the best approach when dealing with a young patient because of the expected long duration of cancer risk, and the fact that surveillance could fail (6). Furthermore, the results of ileal pouch-anal anastomosis are better in young patients. Although "prophylactic colectomy" theoretically eliminates the cancer problem, several factors have made this generally unacceptable. Patients with extensive colitis for more than eight to 10 years are often asymptomatic or have mild disease. Such patients often find it difficult to accept the risks associated with major surgery, the possible morbidity associated with mobilizing the rectum, or the social implications of a permanent ileostomy (in the event a pelvic pouch fails). Besides, most studies suggest that prophylactic colectomy would have been unnecessary in the majority of patients, because they would not have developed carcinoma (3,7).

The third option is colonoscopic surveillance with biopsy. The strategy of such surveillance is either identification of a marker that signals a subgroup of colitics at greatest risk for the development of carcinoma or detection of carcinoma in its earliest recognizable and curable phases (3,7,10,14). Currently, recognition of dysplasia in surveillance biopsy specimens is used as such a marker. Dysplasia, the presumed precancerous epithelial change, has been regularly recognized in colectomy specimens both adjacent to and distant from colitis-associated carcinomas (14,15). Circumstantial evidence suggests that dysplasia may not only be a marker for carcinoma, but may itself be the carcinoma in an early pre-invasive phase (1).

Dysplastic epithelium can occur in a grossly flat mucosa, in a mucosa with a villous or plaque-like configuration, or in a nodular growth resembling adenoma (14-19). Dysplasia is recognized by histologic examination of biopsy specimens utilizing well-defined cytologic criteria that include nuclear enlargement with hyperchromasia, increased mitotic figures, and decreased intracellular mucin (1,14). Most colitis-associated dysplasias resemble adenomas as seen in the noncolitic patient. The pathologist should use the term dysplasia only as a synonym for intraepithelial neoplasia and not in reference to reactive or reparative changes seen with active inflammation. The distinction of repair from dysplasia can be difficult, requires some experience, and in certain instances can be impossible. In general, cytologic abnormalities seen in the presence of active inflammation must be interpreted with caution (1,7). Table 1 compares and contrasts histologic features of dysplasia and repair.
TABLE 1 - DYSPLASIA VERSUS REPAIR: COMPARISON OF HISTOLOGIC FEATURES

<table>
<thead>
<tr>
<th>Feature</th>
<th>Dysplasia</th>
<th>Repair</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear enlargement</td>
<td>+ to +++</td>
<td>+</td>
</tr>
<tr>
<td>Nuclear hyperchromasia</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Nuclear pleomorphism</td>
<td>+ to +++</td>
<td>0 to +</td>
</tr>
<tr>
<td>Nuclear crowding</td>
<td>++ to +++</td>
<td>0 to +</td>
</tr>
<tr>
<td>Irregular nuclear contour</td>
<td>+ to +++</td>
<td>0 to +</td>
</tr>
<tr>
<td>Chromocenters/Nucleoli</td>
<td>0 to +</td>
<td>++ to +++</td>
</tr>
<tr>
<td>Nuclear stratification</td>
<td>0 to +++</td>
<td>+</td>
</tr>
<tr>
<td>Loss of nuclear polarity</td>
<td>0 to ++</td>
<td>0</td>
</tr>
<tr>
<td>Increased mitoses</td>
<td>+ to +++</td>
<td>++</td>
</tr>
<tr>
<td>Inflammatory milieu</td>
<td>+ to +</td>
<td>++</td>
</tr>
<tr>
<td>Decreased intracellular mucin</td>
<td>0 to +++</td>
<td>++</td>
</tr>
<tr>
<td>High nuclear to cytoplasmic size ratio</td>
<td>++ to +++</td>
<td>0 to +</td>
</tr>
<tr>
<td>Cytoplasmic eosinophilia</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Distortion of mucosal architecture</td>
<td>0 to +++</td>
<td>+ to +</td>
</tr>
<tr>
<td>Villous configuration</td>
<td>0 to +++</td>
<td>0</td>
</tr>
</tbody>
</table>

There are many overlaps and no one histologic feature is absolute in making the distinction. Both dysplasia and repair are associated with nuclear enlargement and hyperchromasia, increased mitotic figures, and decreased intracellular mucin. Some features favor repair over dysplasia. Although cells undergoing repair demonstrate nuclear enlargement with hyperchromasia, these nuclei are often evenly spaced and not crowded, round to oval with a smooth external contour, contain granular chromatin with single or multiple chromocenters/nucleoli, and are remarkably similar to one another in size and appearance. In contrast to dysplasia, the nuclear to cytoplasmic size ratio of regenerative cells is often actually decreased, especially in cells adjacent to ulcerated areas. During this phase, the cell cytoplasm is often intensely eosinophilic. Nearby crypt abscesses or neutrophils within the epithelium (cryptitis) help to confirm the diagnosis of repair. As a general rule, reparative epithelial atypism is limited to or accentuated in the crypt base and will not extend into the surface of the crypt.
Features which favor dysplasia over repair are variable nuclear hyperchromasia associated with **PLEOMORPHISM**, irregular nuclear contours, marked nuclear stratification often with irregular nuclear crowding and overlap, and loss of nuclear polarity. The changes of dysplasia will usually extend onto the surface of the crypt. Major distortions of mucosal architecture also favor dysplasia.

*The Inflammatory Bowel Disease - Dysplasia Morphology Study Group* has proposed a three-tiered classification for biopsy interpretation in inflammatory bowel disease: positive, negative, and indefinite for dysplasia (1). My experience has shown this classification to be useful and reasonably reproducible (20). Biopsy specimens negative for dysplasia include normal colon and those showing changes of active or quiescent UC. Positive biopsy specimens are reported as showing either high-grade dysplasia or low-grade dysplasia. Most dysplasias histologically resemble adenomas seen in the noncolitic; however, some do not (15). Dysplasias in this latter group are easy to miss.

In low-grade dysplasia, nuclear changes such as crowding, pleomorphism, hyperchromasia and increased mitotic rate are present, but in general, the nuclei are limited to the basal half of the cell. Usually intracellular mucin is decreased, but on occasion mucin can be increased. Dystrophic or signet ring-type goblet cells are quite common in dysplasia though not pathognomonic. The general mucosal architecture is usually not greatly disturbed.

The distinction between low-grade dysplasia and high-grade dysplasia is made largely on **THE DEGREE OF CYTOLOGIC CHANGE PRESENT**. In high-grade dysplasia, hyperchromasia and pleomorphism are more marked. Stratification with loss of nuclear polarity is often present. Nuclei may be found in the luminal half of the cells. High-grade dysplasia is often associated with distortion of mucosal architecture, most commonly taking the form of a villous growth similar to villous adenoma in the noncolitic.

Biopsy specimens are classified as showing changes indefinite for dysplasia when cytologic abnormalities are seen but these are of insufficient degree to warrant a diagnosis of "true dysplasia." Indefinite changes are usually encountered in a background of active inflammation in which atypical epithelial changes may represent repair or regeneration rather than dysplasia. The indefinite category also includes odd patterns of epithelial growth that have not yet been observed to give rise to carcinoma (e.g., hyperplastic polyp-like change, epithelial changes that resemble gastric foveolar epithelium). This category, indefinite for dysplasia, is a legitimate diagnosis alerting the treating physician that worrisome cytologic changes are present that may place a patient in a higher risk category requiring more frequent surveillance. *The Inflammatory Bowel Disease - Dysplasia Morphology Study Group* subdivides indefinite dysplasia into three groups; probably inflammatory, probably dysplastic, and unknown (1). Obviously, in a category that is already uncertain, this subcategorization is cumbersome, subjective, and associated with marked inter- and intraobserver variation. Therefore, subdivision of biopsy specimens in the indefinite category is not recommended.

**Colonoscopic Surveillance in Ulcerative Colitis**

Patient management recommendations based upon colonoscopic surveillance with biopsy are outlined in Table 2.
**TABLE 2 - ULCERATIVE COLITIS AND DYSPLASIA:**
MANAGEMENT RECOMMENDATIONS BASED ON SURVEILLANCE BIOPSY

<table>
<thead>
<tr>
<th>Biopsy Interpretation</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Regular follow-up</td>
</tr>
<tr>
<td>Indefinite</td>
<td>Short-term follow-up</td>
</tr>
<tr>
<td>Positive low-grade dysplasia</td>
<td>Short-term follow-up, consider colectomy*</td>
</tr>
<tr>
<td>Positive high-grade dysplasia</td>
<td>Consider colectomy*</td>
</tr>
</tbody>
</table>

*Dysplasia must be confirmed (see text)*

Patients whose biopsy specimens remain negative for dysplasia can probably safely continue regular surveillance. Most authorities recommend annual total colonoscopic examination for patients with extensive colitis who have had disease for more than seven to ten years (1,14,17,20). The surveillance interval may be increased to 2 or 3 years for patients with negative initial colonoscopy because only 2% to 4% have been shown to progress (6). Patients demonstrating epithelial changes indefinite for dysplasia require shorter-term follow-up (eg. 6 months to 1 year) (5,21). They must not be ignored because 28% of patients with initial biopsy results that were indefinite for dysplasia progressed to high grade dysplasia and 9% progressed to cancer (6). Management recommendations for patients with low-grade dysplasia in a “flat” mucosa are difficult to make because of the paucity of long-term follow-up information available in this group. Some consider it safe to continue short-term follow-up (eg. three to six months) for patients with low-grade dysplasia (5,21,21a); but if dysplasia persists (17,22) or is associated with any suspicious gross intraluminal lesion or stricture (DALM), colectomy should be considered (1,3,5,18). Forty-three percent of patients with DALM already had carcinoma in immediate colectomy specimens (6). Some advocate immediate colectomy for low-grade dysplasia (4-6,22a,22b) citing that 19% of patients with low grade dysplasia already had complicating carcinomas, but these data are questioned because of case selection bias (6). If high-grade dysplasia is encountered, then colectomy should be recommended because the risk of concurrent carcinoma is estimated to be 42% (6).

Our experience with surveillance biopsy interpretation has shown that true negatives are rarely interpreted as dysplasia, and dysplasia, especially high-grade dysplasia, is rarely missed (20). However, variations in interpretation do occur (1,17,22-24,24a) and in general confirmation of a biopsy diagnosis is desirable before colectomy (1). Any one or more of the following may be considered as adequate confirmation: finding dysplasia in repeat biopsy from the same site, finding dysplasia in one or more additional sites during the same endoscopic examination, or review and confirmation of the dysplasia interpretation by another pathologist experienced with the classification system (1).

Surveillance endoscopy with biopsy has a number of limitations. Currently no compelling evidence exists proving that surveillance benefits the patient (6,10,17,24,25). Participation in a surveillance program does not guarantee that lethal cancer will not develop (5,6,10,17,22,25,26). Dysplasia is an unusual phenomenon and it is difficult for any one pathologist to acquire a great deal of experience.
Therefore, errors in histologic interpretation can be expected. Dysplasia can be extremely focal and is therefore subject to tremendous sampling error (6,20). Since most authorities consider dysplasia a neoplastic change, it is extremely unlikely that it ever resolves spontaneously. Thus, a clinician should never be lulled into a false sense of security by negative follow-up biopsies once true dysplasia has been identified (7,14).

Though not proved scientifically, it seems highly likely that some patients benefit from a surveillance program, because many carcinomas are detected in early curable phases (5,16,17,21,22,25). In addition, there is a low incidence of carcinoma in patients in which biopsy specimens have remained negative (6,17,21). It is unclear whether this benefit justifies the large cost of surveillance endoscopy (6,27). Finally, surveillance programs are pointless unless the patient complies with the regular surveillance and agrees to colectomy when the endpoint (dysplasia) has been reached (27).

Other Surveillance Markers

Many groups have searched for other specific markers for precancer in ulcerative colitis. Sialomucins predominate in cancer and dysplasia in UC (28,29), but the role mucin histochemistry could play in assessing cancer risk has been debated (30-32). Similarly, results of lectin binding, CEA immunocytochemistry, and immunocytochemical analysis for products of C-myc and ras oncogenes have produced variable results and could not reliably differentiate dysplastic epithelium from reparative epithelium in ulcerative colitis (31-35).

Several investigators have described significant correlations between DNA aneuploidy and dysplasia/carcinoma in chronic ulcerative colitis (23,36-38). However, only 80 to 90% of invasive carcinomas and 50% to 80% of the dysplasias demonstrate DNA aneuploidy. This indicates that DNA analysis by flow cytometry is not sensitive enough to be used alone in a cancer surveillance program. Interestingly, similar studies found that many specimens (6% or more) interpreted as negative or indefinite for dysplasia showed DNA aneuploidy (23,37,38). This finding could be interpreted in a number of ways. It could indicate that the test is not specific for dysplasia or carcinoma and these could represent false positives (38). Technical problems linked to false positives include failure to disaggregate nuclear clumps, prolonged exposure of sample to high temperature, and debris in the sample (38,39). Alternatively, this test may identify a subgroup of patients different from the group identified by histologic dysplasia that are showing objective chromosomal abnormalities in the absence of recognizable dysplasia (38). It is tempting to speculate that this DNA aneuploidy could be used as a marker for some of the carcinomas complicating ulcerative colitis, but it is premature to make these conclusions as there are no adequate prospective or long term follow-up studies available for review. Rubin et al in a small series, appear to confirm abnormal DNA content as a predictor of dysplasia (38). However, it is currently unknown whether this "DNA aneuploid-histology negative" group would develop carcinoma or whether they would also have developed histologic dysplasia before developing carcinoma. In Rubin's report, it appears that the "DNA aneuploid - histology negative" group goes through a recognizable dysplasia step. Others have concluded that the detection of DNA aneuploidy was not useful as a predictor of the presence of concurrent carcinoma in UC (36-38) but these studies may be limited by poor flow cytometric techniques and artifacts. Until large prospective studies determine the usefulness of DNA aneuploidy as a marker for malignancy in ulcerative colitis, histology is still the only reliable marker for cancer in a surveillance program (40,41). It is possible that DNA analysis could be of benefit in that patients with diploid DNA content and no signs of histological dysplasia probably can be examined at longer intervals (37,38). Since approximately 90% of patient would be in this category, longer interval surveillance could save considerable amounts of money and time.
An interesting development in the study of colitis-associated carcinoma is the identification of a germline substitution in the MSH2 (Mut S) gene in a subset of UC patients with high grade dysplasia and carcinoma (42). The MSH2 gene on chromosome 2p is involved in DNA mismatch repair and the maintenance of germline stability. A germline T to C substitution was identified at the -6 intronic splice acceptor site of exon 13 in 14/53 (26%) of patients with UC and high grade dysplasia or carcinoma (vs 11% in UC patients without cancer and 9% of normal controls). Noticing that the substitution is highly prevalent in the general population who do not have cancer, the authors postulate that the defect itself may be phenotypically silent until the mismatch repair system is stressed (stressed by inflammation and repair in UC). These data are exciting in that molecular identification of at risk UC patients could lead to, at the very least, better allocation of resources for surveillance and in the best scenario could replace dysplasia as the marker for colectomy. Unfortunately, these results could not be confirmed by another group (43).

"Adenoma" in the Colitic Patient

A special problem concerns the occurrence of "adenomas" in patients with colitis. Colorectal adenomas and inflammatory bowel disease (IBD) are both relatively common. Therefore, there is probably no reason why both conditions could not coexist. Since most IBD-associated dysplasias resemble adenomas (15,21,44,45, 45a), the distinction in practice is often impossible. Therefore, management of patients with ulcerative colitis and “adenoma” has been controversial. Does the lesion represent a relatively harmless sporadic adenoma or is it a dangerous DALM? In general, a pathologic diagnosis of “adenoma” in a patient with IBD must be carefully evaluated by the treating gastroenterologist or surgeon and must at least prompt further consultation with the pathologist because the lesion could represent IBD-associated dysplasia, an ominous change that implies a substantial risk for the development of carcinoma or for coexisting carcinoma (14,46).

Adenoma vs. DALM: Pathological Approaches

Dysplasia in IBD can be broadly classified as flat (endoscopically undetectable or raised). Raised lesions can be further subdivided endoscopically into adenoma-like (i.e., discrete, well-circumscribed sessile or pedunculated lesions that resemble sporadic adenomas) or non-adenoma-like (i.e., irregular plaques or “mass-like” lesions) (46a). Most experts recommend colectomy for flat high-grade dysplasia and endoscopically raised non-adenoma-like dysplasia lesions. The patient management of UC patients with endoscopically adenoma-like dysplasia is not as clear. The differential diagnosis of adenomas and dysplastic lesions in IBD has been the focus of several publications. Distinction based on pathomorphological features has been proposed (47,48). The endoscopic/histologic criteria used by Schneider and Stolte are summarized in Table 3. Although lesions can be classified using this system, to date there has been no clinical correlative or follow-up study to verify that morphologic distinction based on these criteria are valid. The study of Torres et al. (47) suggested that an admixture of normal and dysplastic glands at the surface of the dysplastic lesion could be used to separate IBD-associated dysplasia from conventional adenomas. There are drawbacks to this study that limit its clinical usefulness. An association with carcinoma or flat dysplasia was definitional for IBD-associated dysplasia in this study. Furthermore, there was very limited clinical follow-up to determine if the distinction based on this feature (admixture of normal and dysplasia at the surface) was clinically relevant in patients not yet known to have other areas of dysplasia/carcinoma. Many authorities believe that admixtures of normal and dysplastic glands are so highly prevalent in sporadic adenomas that this criterion may not be helpful in practice.
Table 3 - Adenoma vs. IBD-associated Dysplasia: Proposed Pathomorphological Criteria*

<table>
<thead>
<tr>
<th>Feature</th>
<th>Adenoma</th>
<th>IBD-dysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroscopic</td>
<td>Circumscribed polyp</td>
<td>Plaque or other unusual shape</td>
</tr>
<tr>
<td>Glands</td>
<td>Regularly, round or oval</td>
<td>Irregular</td>
</tr>
<tr>
<td>Gland size</td>
<td>Equal</td>
<td>Unequal</td>
</tr>
<tr>
<td>Intracellular mucin</td>
<td>Regularly distributed</td>
<td>Variable</td>
</tr>
<tr>
<td>Nuclei</td>
<td>Rod-shaped</td>
<td>Round or variable</td>
</tr>
<tr>
<td>Stroma</td>
<td>Sparse</td>
<td>Variable</td>
</tr>
<tr>
<td>Proliferative zone</td>
<td>Luminal</td>
<td>Basal</td>
</tr>
<tr>
<td>Transition with adjacent mucosa</td>
<td>Sharp</td>
<td>Gradual</td>
</tr>
</tbody>
</table>

* Modified from reference 48

Molecular analysis has been applied in an attempt to resolve this differential diagnostic problem. Studies of APC gene mutations using in vitro synthesized protein assays in ulcerative colitis-associated dysplasia versus sporadic adenoma/adenocarcinoma have yielded conflicting results. Tarmin et al. reported significantly lower (6%) rates of APC mutation in ulcerative colitis-associated dysplasia and carcinoma as compared to sporadic neoplasia (74%) (49). However, Redson et al. reported a prevalence of APC gene mutations in ulcerative colitis-associated dysplasia and carcinoma (50%) that was much closer to that seen in sporadic colorectal neoplasia (50). Fogt et al. suggested that the low frequency of loss of heterozygosity (LOH) in the p16 gene (9p) in adenoma compared with dysplasia in ulcerative colitis combined with the infrequent LOH in APC gene loci in cases of non-polyloid dysplasia in UC may be used in combination to differentiate polyloid dysplasia from adenoma in ulcerative colitis (51). The infrequency of LOH at the von Hippel Lindau gene locus in adenomas versus polyloid dysplasia could also be potentially exploited as a discriminator (52). Odze et al report a similar prevalence of 3p, APC, and p16 mutations in adenoma like dysplasia lesions in ulcerative colitis and sporadic adenomas. However, non-adenoma like dysplasia in ulcerative colitis showed significantly higher proportions of 3p and p16 mutations indicating a different timing of molecular events in these lesions (53). Although new and promising, physicians must remember that these molecular approaches are difficult to do, are not generally available and remain to be validated clinically.

Immunocytochemistry has been used in an attempt to discriminate between adenomas and IBD-associated dysplasia (45,54). p53 over-expression by immunohistochemistry, which generally correlates with mutation of the corresponding tumor suppressor gene appears to be the most promising. p53 gene mutations occur earlier in IBD-associated dysplasia versus conventional adenoma/carcinoma. Therefore, strong p53 over-expression tends to correlate with IBD-associated dysplasia. Unfortunately, only approximately 30% of IBD-associated dysplasias over-express p53 (versus 5% of sporadic adenomas) (45,54). This p53 testing is probably not sensitive enough for use in routine practice. Combining p53 immunoreactivity with beta catenin expression (which exploits the relative low prevalence of APC-gene mutation in IBD-associated dysplasia) or bcl-2 immunocytochemistry (which is more often over-expressed in sporadic adenomas) does not appear to improve the sensitivity or specificity (45,54). Immunocytochemistry is easier to do and more widely available. However, like the molecular approaches outlined above, diagnoses based on immunohistochemistry are yet to be validated by clinical follow-up.
Adenoma vs. DALM: A Practical Guide

Pathomorphologic, molecular, or immunocytochemical testing could eventually separate the relatively harmless sporadic adenoma from the more ominous DALM in patients with IBD but this remains to be proved. In the meantime, there may be enough follow-up information to guide the careful physician facing the dilemma of managing a patient with both an “adenoma” and ulcerative colitis. The most important criterion for stratification is the topographical relationship of the adenoma-like lesion to the colitis. Adenoma-like lesions occurring in areas not affected by colitis histologically or endoscopically have not been associated with a particularly high risk of concurrent or subsequent carcinoma. Therefore, these lesions should be considered conventional adenomas that can be adequately treated by polypectomy alone (45,47,55,56). Management of adenoma-like lesions occurring in areas involved by colitis is more challenging because as many as two-thirds of patients have had concurrent or subsequent invasive carcinoma at follow-up (6,47,55). From a practical standpoint, I believe it is prudent to consider all adenoma-like lesions occurring in areas involved by colitis as IBD-associated dysplasias. Having said that, the diagnosis of IBD-associated dysplasia alone in this unique setting (adenoma-like dysplasia in a macroscopic polyp) may not necessarily be an indication for immediate colectomy (1,21,44,45,47,56-58). Endoscopic polypectomy alone may be adequate treatment providing that careful selection criteria are applied, including: 1) the patient is in the adenoma age group (i.e., > 40 years of age), 2) the adenoma-like lesion is discretely defined macroscopically and can be excised in its entirety, 3) excision of the lesion appeared complete to the endoscopist, 4) no “flat” dysplasia was identified in the colon, and 5) the colon was relatively easy to survey (i.e., compliant patient, no inflammatory polyposis). Current data suggest that the majority of patients managed in this fashion will follow a clinically benign course (44,45,56-58, 58a, 58b). However, these patients must receive careful short-term endoscopic surveillance. A 3-6 month initial surveillance interval that can be increased to 6 months to a year following a negative colonoscopy has been suggested (46a). Colectomy should be recommended for patients not fulfilling these selection criteria (58).

Dysplasia and Carcinoma in Crohn's Disease

Increasing epidemiologic and pathologic evidence suggests that patients with Crohn's disease are also at increased risk for the development of intestinal carcinoma (59-65). Those patients who are less than 21 years old at onset of Crohn's Disease may be at an even higher risk (63). The small bowel carcinomas in patients with Crohn's disease have occurred on average, 20 years after onset of Crohn's disease (45a). Most involve the ileum and almost always occur in areas actively involved by Crohn's disease. The carcinomas are clinically and grossly subtle, and often occur in strictured areas that resemble the inflammatory strictures of Crohn's disease. Small intestinal carcinomas in Crohn's disease tend to be poorly differentiated and are associated with a poor prognosis. Approximately one-fourth of cases have occurred in bypassed or out-of-circuit segments (59,61,64,65).

Colonic carcinomas in patients with Crohn's disease have in general developed after about 20 years of disease (60,61,63,64,66-68). The diagnosis is usually made clinically since a gross intraluminal lesion can often be visualized. The colonic carcinomas have been better differentiated than their small bowel counterparts, with an increased prevalence of mucinous histologic type (60,61,67).

Recent reports using current histologic criteria almost invariably note dysplasia in epithelium adjacent to both small bowel and colonic carcinomas in Crohn's disease (60,61,64-67). Dysplasia distant from carcinomas has also been commonly encountered in specimens exhibiting both colonic carcinoma and Crohn's disease. These features suggest that a dysplasia-carcinoma sequence similar to that proposed for ulcerative colitis also occurs in Crohn's disease (61,67).
Cancer Surveillance in Crohn's Disease

Opinions vary regarding the usefulness of cancer surveillance programs in patients with long-standing Crohn's disease (60,61,65,67,69). We think that endoscopic surveillance would be of limited value in small bowel Crohn's disease (45a, 61,70). The small intestine, in general, is inaccessible by current methods. The current histologic marker, dysplasia, is extremely focal. Since only approximately 80 cases of Crohn's-disease-associated small bowel carcinoma have been reported, with approximately one-quarter of these occurring in out-of-circuit segments, the expected cancer or dysplasia yield of a surveillance program would be quite small.

It is possible that surveillance could benefit an occasional patient with colonic Crohn's disease by detecting dysplasia or early carcinoma (67), but we currently question the safety, the necessity, and the benefit of regular cancer surveillance (70). Colonoscopy may be technically impossible or even dangerous in some patients with Crohn's disease. Retained rectal stumps are often strictured and inaccessible. The absolute number of colon carcinomas in Crohn's disease is also low, so again the expected yield of positive cases in a surveillance program would be small.

Rather than ignoring the cancer risk in Crohn's disease, there are options available for the cautious clinician (69,70). A patient with recrudescence of colitis-like symptoms and a background of long-standing inactive Crohn's disease should be thoroughly investigated for carcinoma (61). Yearly surveillance of an out-of-circuit rectum seems reasonable considering that approximately 20% of the reported cases of large bowel cancer in Crohn's disease have occurred in such segments (69,70). It may, however, be better to advise removal of a defunctionalized rectum, especially if re-anastomosis is not planned, is not possible, or is contraindicated. The presence of dysplasia in a biopsy specimen from a patient with Crohn's disease must alert the physician to the possibility of a coexistent invasive malignancy; and in this situation we would recommend management approaches similar to those proposed for dysplasia in MUC (1,61). Clinicians should also recognize that chronic fistula or anal strictures in Crohn's disease may be complicated by squamous cell carcinoma or adenocarcinoma (66). Any abrupt change in the volume or the nature of a fistula's discharge or the development of an area of induration or mass near a fistula should be investigated with biopsy (70).

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SMALL BOWEL BIOPSY: INTERPRETATION AND SPECIMEN HANDLING

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Specimen Procurement and Processing

Small-bowel mucosal biopsy examination remains one of the most important steps in the evaluation of patients with malabsorption (1). A standard gastroscope is used to obtain duodenal biopsy specimens as distally in the small bowel as possible (2,3). The specimens are adequate to evaluate mucosal disease, and because the biopsy is performed under direct vision, many more specimens can safely be obtained.

The most critical part of small bowel biopsy and interpretation is the proper orientation of the specimen. Ideally, specimens are immediately mounted, mucosa side up, on a solid substance such as filter paper, and then placed into fixative. After processing, the histotechnologist embeds the tissue perpendicular to the mounting material. Proper specimen evaluation requires examination of optimally oriented intestinal villi obtained from the central region of the biopsy specimen. Although serial sectioning is advocated by some (4), step sectioning (obtaining ribbons of sections from at least three levels) is a reasonable alternative (5).

Our standard small-bowel biopsy procedure consists of obtaining 4-6 endoscopic biopsy specimens. The samples are fixed and routinely processed. Three to four step-sectioned slides are obtained; three are stained with hematoxylin and eosin, and one with Alcian blue/periodic acid-Schiff (PAS) combination stain. The Alcian blue/PAS stain is a useful screen for Whipple's disease, foveolar metaplasia in chronic duodenitis, and Mycobacterium avium-intracellulare infection. A trichrome stain can be useful for confirming collagen deposits in ischemia or collagenous sprue. In addition, the iron hematoxylin counterstain used in the trichrome technique facilitates identification of Giardia lamblia (5).

Normal Small-Intestinal Histology

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The normal villus to crypt length ratio approximates 3:1 to 5:1 (6). Inflammatory cells, including plasma cells, are normally present in the lamina propria. Intraepithelial lymphocytes occur in a ratio of approximately 1 lymphocyte per 5 enterocytes (4,6). A brush border is often discernable on the enterocyte. The enterocyte nuclei should be basilar in location and evenly aligned.

Brunner's glands in proximal duodenal specimens have an inconsistent effect on villus architecture (6). In some circumstances, normal-length villi may be encountered overlying Brunner's glands, but usually the villi are shorter and somewhat distorted. Similarly, villi are often short and distorted next to or overlying lymphoid aggregates. Such shortened villi should not be misinterpreted as evidence of celiac sprue.

In general, we subscribe to the philosophy that identification of four normal villi in a row indicates that the villus architecture of the whole biopsy specimen is probably normal (4). This does not mean that biopsy specimens with less than four aligned normal villi should be considered inadequate for evaluation, because even one normal villus in a proximal small-bowel biopsy specimen rules out celiac sprue. Conversely, finding four normal villi in a row does not necessarily rule out focal lesions, although it almost always does (5).

**Patterns of Abnormal Small-Bowel Architecture**

The small-bowel mucosal responses to injury are limited and recognition of a response pattern can be useful in differential diagnosis (Table 1). I use the term "severe villus abnormality" to describe a flat intestinal mucosa lacking villi. Usually this change is diffuse, accompanied by intraepithelial lymphocytosis (more than 40 intraepithelial lymphocytes per 100 enterocytes) (7), and associated with crypt hyperplasia, evidenced by numerous mitoses. The terms "severe villus abnormality" or "flat intestinal mucosa" are preferred to "villus atrophy" because the mucosa in the forms associated with crypt hyperplasia is actually of normal thickness. I use the term "variable villus abnormality" to describe specimens in which the villi are either only focally flat or are less than flat (mild or moderate villus shortening) (4). Many specimens in this category will also show increased numbers of intraepithelial lymphocytes (more than 40 per 100 enterocytes) (7). These changes may be associated with features that suggest a specific diagnosis (e.g., numerous eosinophils, granulomas, parasites) or may be non-specific.

**Entities Associated with a Diffuse Severe Villus Abnormality and Crypt Hyperplasia**

**Celiac Sprue**

Celiac sprue (gluten induced enteropathy, gluten sensitive enteropathy, non-tropical sprue) is a major cause of malabsorption (7a). Almost all adult patients in North America with a severe villus abnormality and crypt hyperplasia have celiac sprue (4). The pathogenesis of celiac sprue involves immunologic injury to the enterocyte associated with gluten ingestion, which is a protein found in cereal grains such as wheat, rye, and barley. Celiac sprue is associated with the major histocompatibility alleles DQA1*0501 and DQB1*0201. This HLA-DQ2 combination is found in 98% of patients (7a). Patients with celiac sprue usually show a quick and dramatic clinical and histologic improvement following removal of gluten from the diet and quickly relapse following its reintroduction (8).

The flat mucosa of CS is associated with increased lymphocytes and plasma cells in the lamina propria and increased intraepithelial lymphocytes. The enterocyte nuclei lose their basilar alignment and become stratified. Neutrophils may be present but are usually not prominent. The histologic abnormality is most severe in the proximal intestinal mucosa and gradually lessens distally. With gluten withdrawal, the abnormalities recede from distal to cephalad in the small intestinal mucosa. Thus, proximal small bowel...
biopsy specimens may remain abnormal for quite some time, even in patients showing marked clinical improvement. Remember, a pathologist does not make the diagnosis of CS. All that can be said is that the specimen contained a severe villus abnormality that is consistent with CS. Definitive diagnosis depends upon demonstration of a suitable clinical presentation, compatible serologic tests (9) (e.g., IgA endomesial antibodies, antibodies to TTG, IgG and IgA - antigliadin antibodies) and small bowel histology (10), clinical and, ideally, histologic response to a gluten-free diet, and relapse following gluten challenge.

Mucosal lesions can be classified into five types (7a, 10b). (The Marsh classification which is more popular in Europe than North America). Type 0, the preinfiltrative lesion, is essentially normal. Type 1, the infiltrative lesion, is characterized by intraepithelial lymphocytosis (at least 40 per 100 enterocytes). The type 2 lesion, which is also known as the hyperplastic lesion, shows a variable villous abnormality with epithelial lymphocytosis. The type 3 destructive lesion represents the classic CS lesion described earlier. The hypoplastic type 4 lesion is considered an atrophic end-stage lesion that is seen in a minority of patients unresponsive to gluten withdrawal; it includes the lesion of collagenous sprue.

The histologic differential diagnosis includes all entities that may cause at least a focal severe villus abnormality: common variable immune deficiency, protein allergies other than gluten, some cases of infectious gastroenteritis (11), rare cases of tropical sprue (12), stasis (13), the Zollinger-Ellison syndrome (4, 14), Crohn’s disease, and nonspecific duodenitis. Clinicopathologic correlation is essential for proper diagnosis. All biopsy specimens should be carefully evaluated for plasma cells, since their absence in common variable immunodeficiency syndrome is easy to overlook. Numerous neutrophils, cryptitis, and crypt abscess formation are usually not part of CS, and entities such as infectious gastroenteritis, Zollinger-Ellison syndrome, Crohn’s disease, nonspecific duodenitis, and stasis syndromes, therefore, should be considered.

Refractory Sprue

If there is no clinical response to a gluten-free diet, before changing your diagnosis, remember the most common cause of unresponsiveness is that the diet is not really gluten free (14). Furthermore, wheat is commonly used as an extender in processed foods and can occasionally be present in seemingly non-cereal-grain products, such as ice-cream, cocoa mixes, instant coffee, and salad dressings. Medications, vitamins and mineral supplements may also contain gluten. If dietary indiscretions are ruled out, patients may have refractory or unclassified sprue (4) which may respond to the administration of corticosteroids. Many demonstrate collagenous sprue in their small bowel biopsy specimen (15) (see below). A minority have some unusual histologic features (e.g., thin mucosa or subcryptal inflammatory infiltrate). Refractory sprue can also be associated with cavitation of mesenteric lymph nodes and hyposplenism (16). Lymphoma must be considered in non-responsive patients and should prompt re-review of biopsy specimens or re-biopsy. Furthermore, there is increasing evidence that some refractory sprue cases may actually be low-grade intraepithelial T-cell lymphomas that cannot be recognized without molecular techniques (17-20).

Other Protein Allergies

Allergic reactions to chicken, soy protein, milk, eggs, and tuna fish have been reported to show a flat mucosa similar to celiac sprue (5). Definitive diagnosis depends upon identifying the offending protein, showing a response to withdrawal from the diet and demonstration of recrudescence of symptoms and pathology with its reintroduction.
**Lymphocytic enterocolitis**

Celiac sprue and other "sprue-like" lesions may be associated with a colonic epithelial lymphocytosis similar to what has been described in lymphocytic colitis (21-23). It is possible that in some patients with true celiac sprue (responsive to gluten withdrawal), occult dietary gluten actually reaches the colon and induces the histologic changes of "lymphocytic colitis". However, approximately 1/2 of the patients with "sprue-like" small bowel lesions and "lymphocytic colitis" have not responded to gluten withdrawal. The term "lymphocytic enterocolitis" has been coined to describe this refractory sprue-like condition associated with colonic epithelial lymphocytosis.

**Entities Associated with a Variable Villus Abnormality and Crypt Hypoplasia**

This type of biopsy specimen has been described in malnourished patients with marasmus and kwashiorkor, in patients with megaloblastic anemia and as a sequela of radiation and chemotherapy. Microvillus inclusion disease also typically causes a variable villus abnormality with crypt hypoplasia. This is an inherited autosomal recessive condition that causes intractable diarrhea in infants. Diarrhea persists despite total parental nutrition and patients rarely survive beyond the age of 2 years. The disease should be recognized so that genetic counselling can be offered. Small bowel biopsy specimens usually show a severe villus abnormality with shortened and hypoplastic crypts. Intraepithelial lymphocytes are usually not increased. Transmission electron microscopy establishes the diagnosis by identifying abnormal microvillus structures at the luminal border of the enterocyte and intracytoplasmic inclusions lined by microvilli in the same cells (24, 25). Microvillus inclusion disease can sometimes be suspected based on light microscopic findings because the abnormal microvillus inclusions will be highlighted as PAS positive inclusions. The inclusions can also be recognized with carcinoembryonic antigen immunostaining. Prominent surface enterocyte CD10 immunoreactivity has also been described in microvillous inclusion disease (26).

**Entities Associated with a Non-Specific Variable Villus Abnormality**

Many diseases are associated with non-specific variable villus abnormalities that are usually not flat (see Table 1). Although some (10%) mucosal biopsy specimens showing this change will be from patients with partially treated or clinically latent celiac sprue, other conditions enter into the differential diagnosis including dermatitis herpetiformis, tropical sprue, infectious gastroenteritis, stasis syndromes, Zollinger-Ellison syndrome, systemic mastocytosis, duodenitis with peptic ulcer disease, autoimmune enteropathy, autoimmune disorders and NSAIDs. Clinical correlation is required (5, 27).
Entities Associated with Variable Villus Abnormalities Illustrating Specific Diagnostic Changes

The specific diagnostic features seen in this group of conditions are outlined in Table 2.

**TABLE 1 - PATTERNS OF ABNORMAL SMALL BOWEL ARCHITECTURE**

I. Diffuse severe villus abnormality and crypt hyperplasia:
   A. Celiac sprue
   B. Refractory or unclassified sprue
   C. Other protein allergies
   D. Lymphocytic enterocolitis

II. Variable villus abnormality and crypt hypoplasia:
   A. Kwashiorkor, malnutrition
   B. Megaloblastic anemia
   C. Radiation and chemotherapeutic effect
   D. Microvillus Inclusion Disease
   E. End stage refractory or unclassified sprue

III. Nonspecific variable villus abnormality, usually not flat:
   A. Changes associated with dermatitis herpetiformis
   B. Partially treated or clinically latent celiac sprue
   C. Infection
   D. Stasis
   E. Tropical sprue
   F. Zollinger Ellison Syndrome
   G. Mastocytosis
   H. Nonspecific duodenitis
   I. Autoimmune enteropathy

IV. Variable villus abnormality with specific diagnostic changes:
   A. Collagenous sprue
   B. Common variable immunodeficiency
   C. Whipple's disease
   D. Mycobacterium avium-intracellulare complex infection
   E. Eosinophilic gastroenteritis
   F. Intestinal lymphoma
   G. Parasitic infestation
   H. Waldenströms macroglobulinemia
   I. Lymphangiectasia
   J. Enteropathy-associated T-cell lymphoma
   K. Abetalipoproteinemia
   L. Acrodermatitis enteropathica
   M. Tufting enteropathy

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### TABLE 2 - ENTITIES ASSOCIATED WITH VARIABLE VILLUS ABNORMALITIES ILLUSTRATING SPECIFIC DIAGNOSTIC CHANGES

<table>
<thead>
<tr>
<th>Entity</th>
<th>Diagnostic Feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagenous Sprue</td>
<td>Thickened subepithelium collagen plate</td>
</tr>
<tr>
<td>Immunodeficiency Syndromes</td>
<td>Nodular lymphoid hyperplasia</td>
</tr>
<tr>
<td></td>
<td>Variable villus abnormality associated with absent or reduced numbers of plasma cells</td>
</tr>
<tr>
<td>Whipple's Disease</td>
<td>Foamy macrophages within lamina propria coarse granular intracytoplasmic PAS positive inclusions.</td>
</tr>
<tr>
<td>Eosinophilic Gastroenteritis</td>
<td>Infiltration of mucosa, muscularis mucosae and submucosa by large numbers of eosinophils.</td>
</tr>
<tr>
<td>Enteropathy associated T-cell lymphoma</td>
<td>Ulcers associated with sprue-like mucosa, transformation into large cell lymphoma histology, clonal gene rearrangement.</td>
</tr>
<tr>
<td>Parasitic Infestation</td>
<td>Identification of organism</td>
</tr>
<tr>
<td>Waldenstrom's Macroglobulinemia</td>
<td>Ectatic mucosal lymphatics filled with eosinophilic material, foamy macrophages in lamina propria.</td>
</tr>
<tr>
<td>Intestinal lymphangiectasia</td>
<td>Diffuse dilated lymphatics within mucosa</td>
</tr>
<tr>
<td>Abetalipoproteinemia</td>
<td>Fat accumulation with vacuolization of enterocytes</td>
</tr>
<tr>
<td>Acrodermatitis Enteropathica</td>
<td>Rod-like fibrillar inclusions within Paneth cells by electron microscopy</td>
</tr>
<tr>
<td>Tufting Enteropathy</td>
<td>Focal surface epithelial crowding, disorganization and tufting.</td>
</tr>
</tbody>
</table>
REFERENCES


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NEUROENDOCRINE PROLIFERATIONS OF THE GUT – BIOLOGICAL DIFFERENCES THAT MAKE A DIFFERENCE: SPORADIC CARCINOID VERSUS CARCINOIDOSIS OF THE STOMACH AND TUBULAR VERSUS INSULAR VERSUS GOBLET CELL CARCINOID OF THE APPENDIX

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Gastric Carcinoid Tumors

Approximately five percent of all gastrointestinal neuroendocrine tumors involve the stomach and most are associated with atrophic gastritis. There are important pathologic, epidemiologic, and prognostic differences between sporadic gastric carcinoids and those associated with hypergastrinemic states such as atrophic gastritis.

Sporadic gastric carcinoids are responsible for almost all examples of carcinoid tumor metastatic from the stomach. The gastric primary tumors often form isolated large masses. Gastric carcinoid tumors may produce 5-hydroxytryptophan, gastrin, or ACTH; however, systemic syndromes (e.g., carcinoid syndrome, cushing syndrome) rarely occur. Histologically, sporadic gastric carcinoids typically have a foregut carcinoid tumor pattern with neoplastic cells forming ribbon-like arrangements. Trabeculae and rosette patterns occasional occur. The sporadic gastric carcinoids generally show a negative argentaffin reaction and are argyrophilic with silver stains. Immunoperoxidase stains are usually positive for the pan-
neuroendocrine markers such as neuron specific enolase, chromogranin, and synaptophysin. Sporadic gastric carcinoids may occasionally contain other immunoreactive peptide hormones and biogenic amines. A number of factors have been associated with metastasis including size (greater than 2 cm), and aggressive histologic features (features of intermediate or high-grade neuroendocrine carcinomas). Electron microscopy can demonstrate variable neuroendocrine granule morphology, but this is usually not required for diagnosis.

The carcinoids associated with atrophic gastritis merit special consideration. Patients with atrophic gastritis manifest achlorhydria or hypochlorhydria. This absence of gastric acid leads to compensatory hypergastrinemia. The high gastrin levels have a trophic effect on gastric endocrine cells (enterochromaffin-like [ECL] cells) causing hyperplasia and small carcinoid tumors. A similar phenomenon can be seen in laboratory animals and even humans treated with proton pump inhibitors. Hypergastrinemia-associated gastric carcinoids are also seen with the Zollinger-Ellison syndrome associated with MEN syndrome type 1.

Histologically, patients with “hypergastrinemic” carcinoid tumor usually have gastric atrophy with reduced or absent gastric glands and extensive intestinal metaplasia. Additionally, near the base of the crypts and glands round to cuboidal cells with round and regular nuclei containing coarsely granular chromatin proliferate. These cells show a tendency to nest in an “endocrinoid” fashion and to infiltrate the muscularis mucosae or beyond. The appearance of these cells is typical of ECL-cell hyperplasia, microcarcinoids, and carcinoid tumors that frequently coexist with atrophic gastritis and hypergastrinemia.

The distinction between ECL-cell hyperplasia and carcinoid tumor is arbitrary. Some authors suggest that ECL-cell nodules greater than 1 cm be considered carcinoid tumors. Another proposed histologic classification of gastric endocrine cell proliferation occurring in the setting of chronic atrophic gastritis differentiates hyperplasia, dysplasia, and neoplasia. The hyperplasias (defined as five or more endocrine cells in a chain or cluster) encompass growths up to 150 microns in diameter. Dysplasia described growths measuring 150 microns to 0.5 mm. Lesions greater than 0.5 mm are considered carcinoid tumors and are further subclassified as intramucosal or invasive. In practice, carcinoid tumors arising in the setting of gastric atrophy, hypergastrinemia and ECL-cell hyperplasia rarely metastasize.

Treatment of atrophic gastritis with ECL-cell hyperplasia and carcinoids remains controversial. Some have promulgated endoscopic management with removal of larger carcinoids. Subtotal gastrectomy has been used and recently several successful treatments with antrectomy alone have been reported. Isolated antrectomy represents the most intellectually satisfying treatment strategy since it ideally removes the gastrin-producing cell. In the absence of the trophic factor (gastrin), reversal of the ECL-cell hyperplasia and disappearance of carcinoids have occurred.

**Neuroendocrine Cell Proliferations/Neoplasms of the Vermiform Appendix**

Discussion of appendiceal neuroendocrine neoplasms must begin with a description of the phenomenon referred to as fibrous obliteration/appendiceal neuroma. Fibrous obliteration occurs commonly with a prevalence of nearly 30%. Microscopically, fibrous tissue, chronic inflammatory cells, and neuronal and neuroendocrine cell proliferations obliterate the appendiceal lumen. The latter two can be highlighted by S100 and pan-endocrine immunostaining. Interestingly, “appendiceal carcinoid” often coexists with fibrous obliteration. Some believe “appendiceal carcinoid’s” excellent prognosis (versus other gut carcinoids) relates to the fact that many reported as carcinoids may in fact be exaggerated neuroendocrine cell hyperplasia seen in an otherwise typical fibrous obliteration/appendiceal neuroma. Currently, we require the following for a diagnosis of appendiceal carcinoid; 1) a collection of tumor cells
demonstrating an insular of tubular growth pattern with extension of cells into or through the appendiceal muscular wall or 2) a proliferation of neuroendocrine cells producing a gross nodule or expansion of the appendiceal wall.

Having described minimum criteria for the diagnosis of appendiceal carcinoid, three variant types based on histologic and clinical features can be recognized: Insular carcinoid, tubular carcinoid, and goblet cell carcinoid (synonyms: microglandular carcinoma, goblet cell carcinoma, crypt cell carcinoma, adenocarcinoid, composite carcinoid, mucinous carcinoid).

Insular carcinoid consists of nests or sheets of polygonal cells containing a round to oval nuclei with stippled chromatin. The cytoplasmic cell borders are usually indistinct. The cytoplasm often stains eosinophilic and is sometimes granular. The neoplastic cells of tubular carcinoid arrange in small, well-organized tubules and trabeculae. Some of the tubular lumens may contain mucus. The neoplastic cells are cuboidal with a peripherally placed round nucleus with stippled chromatin. The neoplastic cells usually demonstrate positive immunostaining for CEA and variable staining for chromogranin. The insular and tubular carcinoids of the appendix usually measure less than 1 cm in greatest cross dimension, occur near the appendiceal tip, and generally act in a benign fashion. Right hemicolectomy is recommended for carcinoid tumors larger than 2 cm or for incompletely excised tumors.

Goblet cell carcinoid has features intermediate between insular carcinoid and well-differentiated adenocarcinoma. Goblet cell carcinoids typically infiltrate the appendiceal wall causing a grossly subtle thickening. Mucosal involvement is frequently limited to a proliferation around the base of the crypts. Goblet cell carcinoid infiltrates as small uniform nests or strands or tumor cells. Most cells resemble goblet cells with prominent intracytoplasmic mucin and a crescent-shaped nucleus located at the cell’s periphery. Neuroendocrine cells are frequently absent or in the minority and the typical immunostaining profile shows diffuse positive staining for CEA with only scattered chromogranin positive cells. It is important to recognize goblet cell carcinoid because the prognosis (5 year survival of 80%) is worse than insular and tubular carcinoids (5 year survival of 95%) but is far better than invasive adenocarcinoma (5 year survival of 50%). Right hemicolecotomy is the preferred treatment for appendiceal goblet cell carcinoids that have penetrated the muscularis externa of the appendix, for incompletely resected tumors, and for those tumors having greater than 2 mitoses per 10 high magnification fields. (Although this may relate to coexisting adenocarcinomas-see below).

A common diagnostic pitfall involves invasive adenocarcinoma containing neuroendocrine cells or showing areas of carcinoid tumor. Many invasive adenocarcinomas of the appendix, colon and rectum contain small numbers of neuroendocrine cells. As a general rule, if any part of the tumor has an infiltrative pattern and cellular morphology typical of intestinal adenocarcinomas, they behave like adenocarcinoma and should not be diagnosed as carcinoids or adenocarcinoids simply because they contain scattered focal carcinoid-like areas of neuroendocrine cells.

Incomplete surgical excision of appendiceal neoplasms represents a major indication of right hemicolecotomy. Since neoplasms of the appendix are frequently discovered incidentally during microscopic evaluation of the specimen, we recommend routine sampling of the resection margin.
REFERENCES

DIFFERENTIAL DIAGNOSIS OF GASTROINTESTINAL STROMAL TUMORS (GISTs) IN A GLEEVEC WORLD

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Introduction

For many years, spindle cell neoplasms of the gastrointestinal tract were classified as smooth muscle tumors (i.e., leiomyoma, leiomyosarcoma, or leiomyoblastoma) (1, 2). Ultrastructural and immunohistochemical studies rarely confirmed smooth muscle differentiation and some studies actually concluded that a minority of these tumors were neural in origin or differentiation (1). In 1983, the term “stromal tumors” was introduced by Mazur and Clark (3). The observation that GISTs express the tyrosine kinase c-KIT (CD117) and CD34 (which decorates many of the KIT receptors) provided an important clue to the possible cell of origin or differentiation (4, 5). The interstitial cells of Cajal (ICC) are dendritic-like cells that are widely distributed through the muscularis externa of the GI tract. These cells play an important role in the coordinated contraction of the muscularis externa and have been shown to express both CD117 and CD34. The hypothesis that GISTs are related to ICCs is now widely accepted (4, 6). The link to ICC, being related to both smooth muscle and nerve, helps to explain the historical observations that hypothesized a relationship between GISTs and both smooth muscle and nerve. The expression of KIT is caused by activating KIT gene mutations primarily in exons 9, 11, 13 and 17 (7).

Gleevec (a.k.a. Imatinib Mesylate, STI571, Glivec)

Gleevec is an inhibitor of a specific protein tyrosine kinase that was originally targeted toward platelet-derived growth factor (PDGF) receptor (7, 8). Gleevec inhibits the BCR-ABL fusion product arising from the Philadelphia chromosome of chronic myelogenous leukemia and c-Kit (CD117) of GISTs. The effectiveness of Gleevec in treating CML and metastatic GIST and its relative lack of side-effects led to FDA approval in 2002. Gleevec reduces the size of metastatic deposits in GISTs with post-treatment tumors histologically showing marked decrease in cellularity, hemorrhage and myxoid degeneration (9-11). Side effects are typically minimal (e.g., periorbital edema, nausea, diarrhea, myalgia, fatigue, rash). The most serious side effect gastrointestinal or intra-abdominal hemorrhage apparently occurs in only 5% of patients and is associated with very large tumors (11).
**Diagnosis and Prognosis of GIST**

The diagnosis of GIST should be entertained for all mesenchymal tumors involving the muscularis externa of the gastrointestinal tract and should also be considered in spindle cell neoplasms involving other abdominal sites. Use of an immunohistochemical panel including CD117, CD34, smooth muscle actin, desmin, cytokeratin, S100 protein and HMB45 can be extremely useful in classifying such tumors. More than 96% of GISTs demonstrate KIT by immunohistochemistry and there remains an interesting controversy as to whether GIST can ever be diagnosed in the absence of KIT (4, 6, 12, 13). KIT gene mutation analysis may be helpful in this setting. Some authors believe that it is possible for KIT negative “GIST” to respond to Gleevec. Recently, Heinrich et al and Hirota et al have described PDGFRA mutations in GISTs containing activated but wild type KIT (14, 15).

In the past, I tried with varying degrees of success to predict behavior in GISTs based on such features as size, mitoses counts, subjective assessments of cellularity and the consideration of other morphological features such as infiltration of the mucosa, loss of the perinuclear vacuole, necrosis, nuclear atypia, epithelioid differentiation (in small bowel tumors), and loss of organoid arrangement (in small bowel tumors) (16-22). My experience was that a few tumors were obviously malignant, based on concurrent metastasis, large size and high mitotic counts. Some I considered benign (low cellularity gastric tumors < 5 cm. with mitoses counts of less than 5 per 50 high magnification field or low cellularity, non-epithelioid small bowel < 2 cm. with less than 5 mitoses per 50 high magnification fields with preserved organoid arrangement). Most seem to be of indeterminate biologic behavior.

Criteria for distinguishing benign from malignant GISTs have been described, analyzed and debated for years. Factors such as mucosal invasion, tumor necrosis and high cellularity were statistically associated with malignant behavior but were criticized as subjective and not reproducible. Others have looked at cell proliferation markers such as Ki-67, MIB-1, PCNA, DNA cell cycle abnormalities, and assessment of nucleolar organizing region, however, none of these have been proven useful (1). Size and the number of mitoses figures has been fairly consistent in almost all papers (1). In general terms, I have now embraced the consensus approach to the classification of GISTs (see Table). Currently, it is the KIT status rather than our attempts at prognostication that are paramount because KIT positivity will allow use of Gleevec on label in the event that metastases develop.

<table>
<thead>
<tr>
<th>RELATIVE RISK</th>
<th>SIZE</th>
<th>MITOTIC COUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low risk</td>
<td>&lt; 2 cm.</td>
<td>&lt; 5/50 HMF</td>
</tr>
<tr>
<td>Low risk</td>
<td>2-5 cm.</td>
<td>&lt; 5/50 HMF</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td></td>
<td>6-10/50 HMF</td>
</tr>
<tr>
<td></td>
<td>5-10 cm.</td>
<td>&lt; 5/50 HMF</td>
</tr>
<tr>
<td>High risk</td>
<td></td>
<td>&gt; 5/50 HMF</td>
</tr>
<tr>
<td></td>
<td>&gt; 10 cm.</td>
<td>Any mitotic count</td>
</tr>
<tr>
<td></td>
<td>Any size</td>
<td>&gt; 10/50 HMF</td>
</tr>
</tbody>
</table>
DIFFERENTIAL DIAGNOSIS

**Fibromatosis (Desmoid Tumor)**

Fibromatosis typically occurs in the abdominal wall, mesentery and retroperitoneal tissues and these tumors can attach to, grow into and even through the muscularis externa anywhere in the GI tract (23). Histologically, desmoids are composed of spindled or stellate cells arranged around evenly spaced blood vessels showing a collagenous background. These tumors may have mitoses figures but nuclear pleomorphism is generally absent. Infiltration of the spindle cells at the tumor-mesentery interface favors fibromatosis over GIST. Surprisingly, immunocytochemistry may not necessarily resolve the diagnostic dilemma because CD117 can be positive in fibromatosis although the positivity varies with the antibody used (24-26). Immunopositivity with beta-Catenin protein favors a diagnosis of fibromatosis (27). Interestingly, clinical trials of STI-571 (Gleevec, Glivec) in the treatment of fibromatosis are underway (28).

**Inflammatory Myofibroblastic Tumor**

Inflammatory myofibroblastic tumor encompasses a number of unusual lesions that show a proliferation of spindle cells admixed with chronic inflammatory cells (29). Some of these lesions are clearly benign inflammatory conditions but others have been shown to be clonal and on rare occasions have behaved in a malignant fashion (inflammatory fibrosarcomas). Histologically, inflammatory myofibroblastic tumors are composed of elongate spindle cells and inflammatory cells. Immunostains for desmin and actin are typically positive in inflammatory myofibroblastic tumors whereas CD117 and CD34 are reported as negative.

**Smooth Muscle Tumors**

True smooth muscle tumors can occur in the gastrointestinal tract with most arising in the muscularis mucosae (leiomyoma) (30) or muscularis externa (usually leiomyosarcoma) of the esophagus, colon, rectum and anus (31-33). The lesions typically stain positively for desmin and smooth muscle actin and negative for CD117 and CD34.

**Schwannomas**

Schwannomas of the GI tract are rare and have been most often described in the stomach (34). A peripheral cuff of lymphoid aggregate, nuclear palisading and hyalinized blood vessels can be useful in suggesting the diagnosis. Schwannomas stain for S100 protein and are CD117 negative. CD34 positivity can be variable.

**Solitary Fibrous Tumor (Submesothelial Fibroma)**

Solitary fibrous tumor can occur anywhere in the peritoneal cavity and can adhere to the bowel. They are typically highly cellular spindle cell proliferations with depositions of collagen that usually have very few mitoses figures. These tumors are CD34 positive and can easily be confused with GISTs. Solitary fibrous tumors are negative for CD117 (29, 35).
REFERENCES