Morphologic Criteria of Invasive Colonic Adenocarcinoma on Biopsy Specimens
C Rose, H Wu

Citation

Abstract
The World Health Organization (WHO) defines an invasive colonic adenocarcinoma as a tumor that invades through the muscularis mucosa, into or beyond the submucosa. This feature may not be easily distinguishable in scant colonoscopic biopsy specimens. Our objective is to identify additional morphologic criteria to aide in the diagnosis of invasive colonic adenocarcinoma on scant biopsy material. We evaluated six additional morphologic criteria in a retrospective observational study of 55 consecutive malignant and 16 benign colonic biopsy specimens whose final diagnoses were confirmed by concordant colonic resection. Statistic analysis shows that desmoplasia, cribiform pattern, intraluminal necrosis and high-grade nuclear atypia are independent factors that are highly associated with invasive adenocarcinoma (p < 0.05). Both desmoplasia and cribiform pattern demonstrate 100% specificity and high sensitivity with 83.6% and 89.1% respectively in diagnosing invasive colonic adenocarcinoma. Intraluminal necrosis and high-grade nuclear atypia also showed high sensitivity (92.7% and 90.9%) and high specificity (93.8% and 87.5%). The presence of any of the following four histologic features: stromal desmoplasia, cribiform pattern, intraluminal necrosis and high-grade nuclear atypia in the biopsy specimen, should warrant consideration of a complete resection.

INTRODUCTION
Colonic adenocarcinoma is the third most common cause of cancer mortality in the United States. As such, screening colonoscopies are utilized to detect colon cancer at its earliest and potentially curable stage, resulting in a surge in the number of colon biopsies submitted to pathology departments. On biopsy specimens, the diagnostic criteria for colonic adenocarcinoma are different between Japanese and Western pathologists. According to the WHO definition, colonic adenocarcinoma is diagnosed when neoplastic glands invade submucosa and beyond. However, biopsy specimens have a propensity to be small and poorly oriented, causing difficulty in the demonstration of invasion to the submucosa. Other than invasion to the submucosa, many pathologists rely on the presence of desmoplasia as an additional criterion to diagnose invasive adenocarcinoma. An invasive adenocarcinoma consistently induces a desmoplastic reaction and desmoplasia was noted to be highly associated recurrent cancer in patients with endoscopic resection of submucosal invasive adenocarcinoma (pT1). We attest there are a group of additional morphologic features that can aide in the diagnosis of invasive colonic adenocarcinoma in biopsy material and that these evidence based features should be considered as additions to the current criteria.

MATERIALS AND METHODS
This study is a retrospective observational analysis of colon biopsy specimens and their subsequent resections that occurred at a community hospital in 2008. A total of 71 cases were recruited including, 16 benign and 55 malignant cases based on the resection results. It was key that each biopsy evaluated have a subsequent resection in order to decrease the effect of sampling error on the data. Each specimen had the following demographic information gathered: age, sex, and site. The biopsy specimens had the following evaluated: the volume of biopsy, and six morphologic features including the presence/absence of desmoplastic stromal response (Figure 1), the presence/absence of a cribriform pattern, the presence/absence of atypical glands penetrating the muscularis mucosa (Figure 2), the presence/absence of atypical glands next to the normal mucosa (Figure 3), the presence/absence of intraluminal necrosis (Figure 4) and the presence/absence of high grade nuclear atypia. Cribriform pattern is defined as gland within gland and/or back-to-back arrangement without stroma in between (Figure 3). High-grade nuclear atypia is...
defined as rounded or pleomorphic nuclei with prominent nucleoli and high nuclear to cytoplasmic ratio as well as loss of the nuclear polarity (Figure 4). Atypical gland is defined as a gland with abnormal complex architecture and nuclear atypia.

The morphologic features were assessed independently by two pathologists and the final consensus results were achieved by double headed scope discussion. The features were analyzed using Fisher's Exact Test, Pearson Chi-Square and Goodman and Kruskal’s Lambda directional statistic.\(^6\) The sensitivity, specificity and positive predictive value were also calculated. This study was approved by the Institutional Review Board of Indiana University Health Ball Memorial Hospital.

**Figure 1**

Fig 1. Invasive colonic adenocarcinoma with desmoplastic stromal response, H&E stained x 200

**Figure 2**

Fig 2. Invasive colonic adenocarcinoma glands penetrating muscularis mucosa, H&E stained x200

**Figure 3**

Fig 3. Abnormal colonic glands with back to back and cribriform arrangement adjacent to normal glands, H&E stained x200
**RESULTS**

The number of biopsy per case ranges from 1 to 12 (mean 4.9) for benign cases and 1 to 28 (mean 6.6) for malignant cases. There were 5 males and 11 females with age ranging from 42 to 84 years in benign cases and 23 males and 32 females with age ranging from 34 to 92 years in malignant cases. The tumor sites for malignant cases include right colon 29 cases, left colon 21 cases and transverse colon 5 cases and the tumor sites for benign cases include right colon 9 cases, left colon 4 cases and transverse colon 3 cases, (Table 1).

The final diagnoses in resection specimens for benign cases were 11 cases of adenomas, 5 cases of Crohn’s disease and 1 case of ischemic colitis. In all 16 cases the biopsy diagnoses were in agreement with the final diagnoses in the resection specimens. The final diagnoses in resection specimens for malignant cases include 49 cases of low-grade adenocarcinoma and 6 cases of high-grade adenocarcinoma. In 46 of 55 malignant cases the initial biopsy specimens were diagnostic for invasive adenocarcinoma. In the remaining 9 cases the biopsy diagnoses were adenoma (2 cases), high-grade dysplasia (3 cases) and suspicious for adenocarcinoma (4 cases).

Analyzed by Pearson chi-square and Fisher’s exact tests, all the six morphologic features including desmoplastic stromal response, cribriform pattern, atypical glands next to the normal tissue, atypical glands penetrating muscularis mucosa, intraluminal necrosis and high grade nuclear atypia were shown to have a statistically significant association with invasive colonic adenocarcinoma (p-value < 0.001), see Table 2.

**Figure 5**

Table 1: Evaluations of the 6 histologic features in the biopsy specimens of benign and malignant colon masses

<table>
<thead>
<tr>
<th>Features</th>
<th>Chi-Square</th>
<th>Exact Test</th>
<th>Kruskal’s Lambda Directional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desmoplasia</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>Cribriform</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.012</td>
</tr>
<tr>
<td>Penetrating muscularis mucosa</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.042</td>
</tr>
<tr>
<td>Atypical glands next to normal</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.030</td>
</tr>
<tr>
<td>Intraluminal necrosis</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>High grade nuclear atypia</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.005</td>
</tr>
</tbody>
</table>

However, when controlling all the covariate and considering the dependent variable, Goodman and Kruskal’s Lambda directional statistics showed that invasive colonic adenocarcinoma is most significantly associated with desmoplasia and intraluminal necrosis, followed by high grade nuclear atypia and cribriform pattern (p-value < 0.05). Atypical glands penetrating muscularis mucosa and atypical glands next to the normal mucosa are not as associated as other variables. Both desmoplasia and cribriform pattern have 100% specificity and positive predictive value and
reasonably high sensitivity with 83.6% and 89.1% respectively. Intraluminal necrosis and high-grade nuclear atypia also showed high sensitivity (92.7% and 90.9%), high specificity (93.8% and 87.5%) and high positive predictive value (98% and 96%), see Table 3.

**DISCUSSION**

There are no consensus diagnostic criteria for invasive adenocarcinoma in colon biopsies. There are vast discrepancies in the diagnosis of gastrointestinal adenomas and adenocarcinomas between Western and Japanese pathologists due to differences in the diagnostic criteria. In Japan, colonic adenocarcinoma is diagnosed on the basis of glandular structures and nuclear features, whereas Western pathologists require the presence of invasion into the submucosal layer for the diagnosis of colorectal adenocarcinoma which is in accordance with the WHO definition. Due to superficial nature of the colonoscopic biopsy, it can be difficult to demonstrate tumor invasion of the submucosa.

Many pathologists rely on the presence of stromal desmoplasia to diagnose invasive adenocarcinoma, even though the features of tumor invasion into the submucosa are not present in the specimens. Many cases of unequivocal diagnosis of invasive adenocarcinoma in our study were based on the presence of desmoplasia. Poorly-differentiated adenocarcinoma and desmoplasia were noted to be highly associated recurrent cancer in patients with endoscopic resection of submucosal invasive adenocarcinoma. Desmoplasia is the most important criteria for a practicing pathologist to diagnose an invasive low grade adenocarcinoma, in the absence of tumor invasion to the submucosa. In our study, we demonstrate that other than desmoplasia, other morphologic criteria such as cribriform glandular pattern, intraluminal tumor necrosis, and high-grade nuclear atypia are also independent factors that have a high statistical association with invasive adenocarcinoma. (p-value < 0.05) Desmoplasia and cribriform pattern are the most specific criteria; both showed 100% specificity and positive predictive value in our study. However, intraluminal tumor necrosis and high-grade nuclear atypia had higher sensitivity in diagnosing adenocarcinoma at 92.7% and 90.9%. Both atypical glands penetrating the muscularis mucosa and atypical glands next to the normal colon mucosa are not independent factors to be associated with the invasive adenocarcinoma because these atypical glands often form a cribriform pattern and show high-grade nuclear atypia.

In conclusion, the presence of any of the following four histologic features: stromal desmoplasia, cribriform pattern, intraluminal necrosis and high-grade nuclear atypia in the biopsy specimen, should warrant a complete resection of the lesion. These features have a high statistical association with invasive colonic adenocarcinoma. A larger, multicenter study should be conducted to verify our observation. Evidence based results should help to further refine and reduce the differences between the diagnostic criteria for invasive colonic adenocarcinoma between pathologists worldwide.

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**References**

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