Incidence of Diagnostic Change in Colorectal Polyp Specimens After Deeper Sectioning at 2 Different Laboratories Staffed by the Same Pathologists

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ABSTRACT

Objectives: To calculate the incidence of nondiagnostic (ND) colorectal (CR) polyp cases in which deeper tissue sectioning rendered new diagnostic information—particularly adenomas—in 2 laboratories staffed by the same pathologists.

Methods: After initial diagnosis, 100 ND CR polyps from each laboratory were reexamined with 3 deeper levels to establish rates of diagnostic conversion based on biopsy specimen location and original observation(s).

Results: Deeper sectioning rendered new diagnostic information in 43 (21.5%) of 200 biopsy specimens and specifically adenomas in 16 (8.0%) of 200 biopsy specimens.

Conclusions: These results support routine ordering of deeper levels on ND CR polyps to improve adenoma detection rates, especially those cases without any histologic abnormality. If another biopsy in the same case already is adenomatous, examination of deeper levels may not be necessary, as it may not have any significant effect on the clinical management of the patient.

The practice of ordering deeper levels to increase diagnostic accuracy is neither novel nor applicable only to gastrointestinal specimens. Previous studies have shown this technique to be useful in skin,1 esophageal,2 prostate,3 cervical,4 and breast biopsy specimens5 and even in intraoperative frozen sections.6 Because the clinically significant vs completely benign polyps in colorectal (CR) biopsy specimens can be clinically asymptomatic and endoscopically homogeneous, specificity and accuracy in histologic assessment are essential for diagnostic practice and effective patient treatment.7 Among CR polyps, adenomatous polyps are of particular concern because of their malignant potential and subsequent obligation for a more extensive patient follow-up.8 Some polyps, such as serrated and hyperplastic polyps (HPPs), are more common in specific locations of the colon; however,
adenomatous polyps are common throughout the colon.9 The reported rates of detecting adenomatous tissue on deeper sections range from 1.3% to 19.7% of biopsy specimens that are originally nondiagnostic (ND).8,10-12 In an attempt to develop a realistic laboratory protocol that balances precision in diagnosis, convenience for the patient, cost-effectiveness for the laboratory, and time efficiency for the pathologist, previous studies on CR biopsy specimens have considered the value of second opinions,13 explored benefits of step sectioning vs serial sectioning,14 experimented with reorienting specimens 180 degrees to obtain diagnostic information from both sides of a specimen,10 correlated the size of the polyp8 or the presence of a lymphoid aggregate (LA)15 with the potential of discovering an adenoma, and evaluated cost with respect to diagnostic yield obtained from various depths of deeper levels.16 Accordingly, the purpose of the current study was to prospectively determine the incidence of ND CR polyps in a typical population that rendered new diagnostic information—with specific attention to adenomas—on examining deeper levels of the remaining tissue left in the block to establish rates of diagnostic conversion or error in 2 different laboratories staffed by the same 4 pathologists. In addition, the rates of change or unchanged diagnoses were analyzed with respect to their specific location in the colon (ascending, transverse, descending, and rectosigmoid) and original diagnoses (no histologic abnormality [NA], LA, or suggestive of hyperplastic polyp [SHPP]) to assess if either biopsy specimen location or original diagnoses correlate with identifying new significant diagnostic information.

Materials and Methods

From May 11, 2009, through May 27, 2009, the Texas Digestive Disease Consultants (TDDC) Histology Laboratory in Flower Mound, TX, received 1,441 endoscopic CR biopsy specimens designated “polyp,” defined by the submitting gastroenterologist based on his or her endoscopic evaluation. The biopsy specimens were routinely processed and cut. Three levels with 2 H&E-stained sections per level sectioned to at least 50% of the tissue depth were initially diagnosed by 1 of 4 pathologists. If the initial diagnosis was considered ND (non-dysplastic or neoplastic, with or without LA), 3 deeper levels were examined on 1 slide in an attempt to uncover additional diagnostic information. These deeper levels were taken from the original tissue block at approximate depths of 25%, 50%, and 75% into the remaining tissue and placed on 1 slide. All original slides and corresponding deeper levels were blindly reviewed by a second pathologist to confirm both the original and final diagnosis. Following the blind review, all original diagnoses were confirmed, and no cases were excluded from the study due to diagnostic discrepancy. Any CR specimens that were not submitted as a “polyp” or that rendered a specific diagnosis on examination of the original 3 levels were excluded from the study.

Of the 1,441 biopsy specimens, 132 were considered ND (9.2%); of those ND specimens, deeper levels were examined on a subgroup of 100 (6.9%) biopsy specimens from 91 patients. To establish that the workload for the month of the study (May 2009) was typical, we conducted a search of TDDC’s pathology laboratory database for all endoscopic CR biopsy specimens designated clinically as “polyp” and processed from May 2008 through April 2009 (12 months before the study). The diagnoses (ND, adenoma [AD], HPP, and other) of the biopsy specimens were tallied and compared month by month. Diagnostic criteria for diagnosis of AD and HPP were uniform among pathologists, and any dysplasia, even involving a single crypt, was considered sufficient for a diagnosis of AD. A diagnosis of LA was rendered in biopsy specimens with even a single small, round distinct collection of lymphocytes without other specific diagnostic changes. Hyperplastic polyp was diagnosed when serrated architecture was unequivocal. Biopsy specimens with subtle or rare serrated luminal change judged inadequate for a diagnosis of HPP were diagnosed as SHPP. No case originally diagnosed as HPP or SHPP was changed to sessile serrated adenoma or polyp on subsequent retrospective blind review. The monthly rates of biopsies were averaged and compared using standard deviations and ranges of the diagnostic rates during May 2009.

Similarly, from May 8, 2009, through July 8, 2009, the histology laboratory for ProPath Services, LLP in Dallas, TX, received 1,412 endoscopic CR biopsy specimens designated “polyp” by the endoscopist. The specimens were routinely processed, diagnosed by one of the same 4 pathologists, and the criteria for ordering deeper levels were applied as described above. Of the 1,412 biopsy specimens, 223 (15.8%) were considered ND; of those ND specimens, deeper levels were reviewed on a subset of 100 (7.1%) biopsy specimens from 84 patients. All ND biopsy specimens were grouped into 1 of 3 categories based on original observations: NA, LA, or SHPP. The diagnoses with respect to biopsy specimen location (ascending, transverse, descending, or rectosigmoid colon) were tabulated. Significance was determined by calculating the difference between 2 independent proportions (http://vassarstats.net/propdiff_ind.html).

Results

Of the 100 biopsy specimens from TDDC on which deeper levels were obtained, the original interpretation was NA in 42 (42.0%), LA in 39 (39.0%), and SHPP in 19
Fifteen (15.0%) of the 100 biopsy specimens revealed new diagnostic information after evaluation of the deeper levels. Five (5.0%) of these biopsy specimens uncovered LA, 8 (8.0%) were diagnosed as AD, and 1 (1.0%) was diagnosed as leiomyoma of the muscularis mucosae (LMM). No diagnostic change was made in 31 (73.8%) of the 42 biopsy specimens originally interpreted as NA, 28 (90.0%) of the 31 with LA, and 16 (84.2%) of the 19 biopsy specimens suggestive of HPP.

Table 1
Percentage and Category of Nondiagnostic Original and Final Diagnoses After Deeper Levels Examined (Combined Data)

<table>
<thead>
<tr>
<th>Original Diagnoses (n = 104)</th>
<th>Final Diagnoses (n = 59)</th>
<th>SHPP (n = 37)</th>
<th>Total (n = 200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>LA</td>
<td>SHPP</td>
<td></td>
</tr>
<tr>
<td>9.6</td>
<td>11.5</td>
<td>7.7</td>
<td>5.0</td>
</tr>
<tr>
<td>94.9</td>
<td>13.5</td>
<td>8.0</td>
<td>78.5</td>
</tr>
</tbody>
</table>

AD, adenoma; HPP, hyperplastic polyp; LA, lymphoid aggregate; LMM, leiomyoma of muscularis mucosae; NA, no histologic abnormality; SHPP, suggestive of hyperplastic polyp.

Table 2
Original and Revised Diagnoses and Adenoma Detection by Site Within the Colon

<table>
<thead>
<tr>
<th>Location</th>
<th>NA (n = 104)</th>
<th>LA (n = 59)</th>
<th>SHPP (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascending (n = 55; 27.5%)</td>
<td>31 (56.4)</td>
<td>17 (30.9)</td>
<td>7 (12.7)</td>
</tr>
<tr>
<td>Transverse (n = 34; 17.0%)</td>
<td>20 (68.8)</td>
<td>13 (48.2)</td>
<td>1 (3.6)</td>
</tr>
<tr>
<td>Descending (n = 32; 16.0%)</td>
<td>16 (50.0)</td>
<td>10 (31.3)</td>
<td>6 (18.8)</td>
</tr>
<tr>
<td>Rectosigmoid (n = 79; 39.5%)</td>
<td>37 (46.8)</td>
<td>19 (24.1)</td>
<td>23 (29.1)</td>
</tr>
<tr>
<td>Diagnostic change (n = 43; 21.5%)</td>
<td>33 (76.7)</td>
<td>3 (7.0)</td>
<td>7 (16.3)</td>
</tr>
<tr>
<td>Adenoma conversion (n = 16; 8.0%)</td>
<td>12 (75.0)</td>
<td>2 (12.5)</td>
<td>2 (12.5)</td>
</tr>
</tbody>
</table>

LA, lymphoid aggregate; NA, no histologic abnormality; SHPP, suggestive of hyperplastic polyp.
change after examination of deeper levels and 75.0% of adenomas originally were NA.

Of the biopsy specimens with NA as the original diagnosis, 29.8% were from the ascending colon, 19.2% were from the transverse colon, 15.4% were from the descending colon, and 35.6% were from the rectosigmoid colon. Of the biopsy specimens with LA as the original diagnosis, 28.8% were from the ascending colon, 22.0% were from the transverse colon, 17.0% were from the descending colon, and 32.2% were located in the rectosigmoid colon. Of the SHPP biopsy specimens, 18.9% were from the ascending colon, 2.7% were from the transverse colon, 16.2% were from the descending colon, and 62.2% were in the rectosigmoid colon. Of the 43 (21.5%) cases that yielded new diagnostic information on examination of deeper levels, 34.9% were in the ascending colon, and 41.9% were from the rectosigmoid colon. Of the 16 (8.0%) cases that were diagnosed as AD on deeper levels, 50.0% were from the ascending colon.

At TDDC and ProPath combined, there were 104 biopsy specimens without histologic abnormality (NA), 33 (31.7%) of which rendered new diagnostic information on examination of deeper levels, whereas only 10 (10.4%) of the 96 LA and SHPP cases revealed new diagnostic information. Using a significance of the differences of proportions test, biopsy specimens that originally were NA were more likely to uncover new diagnostic information than those with LA or SHPP (2-tailed, $P = .0002$, $z = 3.7$). With regard to location of the biopsy specimen as an indicator of diagnostic conversion, 15 changes from the 55 right colon biopsy specimens were compared with 18 changes from the 79 rectosigmoid colon biopsy specimens. No statistical significance was found ($z = 0.6$).

The monthly rates of diagnosis by classification of 12 consecutive months before the study at TDDC were averaged to determine the mean rate of diagnosis of each category before deeper levels were examined. The mean rates of diagnosis in the 12 months before the month of the study (May 2008 through April 2009) were 11.9% ND, 55.1% AD, 31.4% HPP, and 1.6% other diagnoses. The rates during the month of the study (May 2009) were 9.6% ND, 57.5% AD, 31.1% HPP, and 1.8% other diagnoses, respectively, confirming that the study period was typical and representative.

**Discussion**

Several other studies have confirmed that examination of deeper levels improves diagnostic accuracy in ND CR “polyp” biopsy specimens and subsequently improves patient care. However, we are not aware of another study that compares diagnostic change and adenoma detection rates on examination of deeper levels in ND CR biopsy specimens from 2 different laboratories staffed by the same pathologists, with emphasis on the original diagnosis and biopsy specimen location. We focused on the detection of adenomatous polyps because these precursor lesions are estimated to cause between 70% and 90% of colorectal cancers.

**Table 3**

<table>
<thead>
<tr>
<th>Original Diagnoses, No. (%)</th>
<th>Location</th>
<th>NA (n = 104)</th>
<th>LA (n = 59)</th>
<th>SHPP (n = 37)</th>
<th>Diagnostic Change (n = 43)</th>
<th>Adenoma Conversion (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascending</td>
<td>31 (29.8)</td>
<td>17 (28.8)</td>
<td>7 (18.9)</td>
<td>15 (34.9)</td>
<td>8 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Transverse</td>
<td>20 (19.2)</td>
<td>13 (22.0)</td>
<td>1 (2.7)</td>
<td>7 (16.3)</td>
<td>4 (25.0)</td>
<td></td>
</tr>
<tr>
<td>Descending</td>
<td>16 (15.4)</td>
<td>10 (17.0)</td>
<td>6 (16.2)</td>
<td>3 (7.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Rectosigmoid</td>
<td>37 (36.6)</td>
<td>19 (32.2)</td>
<td>23 (62.2)</td>
<td>18 (41.9)</td>
<td>4 (25.0)</td>
<td></td>
</tr>
</tbody>
</table>

LA, lymphoid aggregate; NA, no histologic abnormality; SHPP, suggestive of hyperplastic polyp.

**Figure 2** Average rates of diagnoses by classification in the 12 months before the study (May 2008 through April 2009) and the month of the study (May 2009). AD, adenoma; HPP, hyperplastic polyp; ND, nondiagnostic.
This study was conducted in 2 different laboratories to examine histotechnologist variability. The slides from routine histology and deeper levels were reviewed by a second pathologist to ensure that all diagnoses were in agreement. All new diagnoses were made by at least the second deeper level, and none of the tissue in the blocks was exhausted even after the third deeper level. Figure 3 outlines the complete review path in the study, beginning with 100 ND CR biopsy specimens from TDDC and ProPath, the original diagnoses, and the final diagnoses. Examination of deeper levels rendered new diagnostic information in 15 (15.0%) biopsy specimens at TDDC and 28 (28.0%) at ProPath; however, interestingly both laboratories had the same adenoma detection rate of 8.0%. Similar studies have reported a conversion rate anywhere from 1.7%\textsuperscript{11} to 36.9%\textsuperscript{16} and adenoma detection rates from 1.29%\textsuperscript{11} to 19.7%,\textsuperscript{10} which are dependent on the design of the study and the standard practices of the laboratory. For example, the Parameswaran et al\textsuperscript{11} study, which recorded a 1.7% diagnostic conversion rate and only a 1.3% adenoma detection rate, excluded using multiple ND biopsy specimens from the same patient to “ensure that each study specimen was biologically independent” and focused on reducing interobserver error as the primary means of increasing diagnostic accuracy (3.9% change). Other factors that could influence these rates include the standard depth of tissue cut in routine histology by histotechnologists of a particular laboratory, the differences in technique among individual technologists’ work in a single laboratory, and the number of both original and deeper levels examined in each laboratory. Each laboratory

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Distribution pattern of the entire material in the study (ProPath and Texas Digestive Disease Consultants [TDDC]) based on original diagnoses from routine sections and new diagnostic information from subsequent deeper levels. CR, colorectal; LA, lymphoid aggregate; LMM, leiomyoma of muscularis mucosae; NA, no histologic abnormality; ND, nondiagnostic; SHPP, suggestive of hyperplastic polyp.}
\end{figure}
in our study prepared 3 levels of 2 sections on both the original slides and the deeper slides.

Of the 3 ND biopsy categories, biopsy specimens that originally showed NA, compared with those having LAs or SHPP changes, were statistically more likely to uncover new diagnostic information, accounting for 76.8% of the diagnostic changes in the study. Although 75.0% of the adenomatous polyp conversions were from biopsy specimens that originally showed no histologic abnormality (NA), the sample size was not large enough to confirm a statistically significant correlation. Nevertheless, our results are consistent with a similar study by Polydorides et al,12 in which higher conversion rates of tubular adenomas were found in biopsy specimens of originally normal colonic mucosa. Comparatively, Wu et al15 commented that examination of deeper levels in 8 (~10%) of 83 biopsy specimens resulted in “polyp” detection, 5 of which were adenomas, for an adenoma detection rate of 6%. Because 5 of the 8 polyps were associated with LAs (3 of the 5 adenomas) on the initial sections, the authors proposed that “the presence of lymphoid aggregates within initial sections fails to exclude the presence of polyps within additional sections.” Originally, our study showed 59 cases with LA (1 and 2 of which became HPP and AD, respectively) and 104 cases without histologic abnormality (8 and 12 of which became HPP and AD, respectively). Therefore, our results confirmed that the presence of an LA did not rule out a polyp in additional sections, but we also found that biopsy specimens originally showing NA were more likely to render a diagnosis of AD than those with LA or SHPP.

Although the raw data seemed to indicate that diagnostic conversion and adenoma detection from deeper levels were more likely in ascending and rectosigmoid biopsy specimens, a statistical correlation could not be made. Fifteen (27.3%) of 55 biopsy specimens were from the ascending colon compared with 7 (20.6%) of 34 from the transverse colon, 3 (9.4%) of 32 from the descending colon, and 18 (22.8%) of 79 from the rectosigmoid colon. Although 15 (34.9%) and 18 (41.9%) of the 43 conversions were from the ascending and sigmoid colon, respectively, this may reflect the fact that our endoscopists submitted more ascending and rectosigmoid biopsy specimens. The descending colon had the lowest incidence of diagnostic change (3 of 43; 7.0%); however, statistical calculations could not be made on the descending colon with confidence because the standard binomial requirement (n > 5) was not satisfied. For the same reason, although 8 of the 16 biopsy specimens with AD on deeper levels were from the ascending colon (50.0%), statistical calculations for adenoma detection could not be performed.

The monthly averages (Figure 2) for the year before the deeper levels study at TDDC demonstrate that the month during which the study was conducted (May 2009) is representative of a typical month. All May 2009 values are within the lowest and highest values for their respective diagnostic category. Most May 2009 values are within 1 standard deviation of the average rates of diagnosis from the preceding 12 months, and all are fully within 2 standard deviations.

Several studies have examined issues of cost-effectiveness and clinical practicality in ordering deeper levels. Considering the cost of the raw materials, a histotechnologist’s time to cut and stain deeper levels, and the pathologist’s time, Nash et al5 concluded that each new adenomatous polyp identified cost approximately $94.90. Polydorides et al12 examined the benefits of reorienting specimens 180 degrees to sample both sides of a block to obtain additional diagnostic information without exhausting the tissue. This study initially calculated $711 per adenoma identified and subsequently recommended further processing of polyps greater than 5 mm, which reduced costs to $291 per adenoma. Wu et al15 examined the optimal depth of leveling using 50 deeper levels to sufficiently investigate originally ND CR biopsy specimens, yet not exhaust the tissue, and concluded that depths of 240 to 260 μm were recommended to uncover 90% of histologically occult polyps. Some would argue that it is not always necessary to make a histologic diagnosis on diminutive colon polyps. One study found that “only 0.9% of diminutive polyps (84 of 9042) contained advanced histology” and calculated that forgoing the pathologic assessment of such specimens would save more than $1 billion upfront without affecting patient care.18

To minimize costs and conserve tissue in our study, we placed the 3 deeper levels on the same slide. In addition, we did not exhaust the tissue so that other stains could be performed on the remaining tissue in the block if needed. However, the economic impact of obtaining the deeper levels was not considered.

We found that all new diagnoses were identified by the second (of 3) deeper levels. Moreover, the tissue was never exhausted by the third deeper level. Therefore, following conclusion of the study, we altered the estimated depth that the histotechnologist sectioned tissue blocks such that the third and final level on an original slide would be at approximately the same depth in the block as the previous protocol’s second (of 3) deeper levels beyond the original slide. Doing so should afford the ability to detect additional pathology, including adenomas, by the third and final routine level on an originally sectioned block and slide while also not exhausting the tissue. Subsequent to this study, our pathologists routinely pursue deeper level sectioning in cases where all CR “polyp” biopsy specimens are ND, especially those without any histologic abnormality (NA). Nevertheless, if another biopsy specimen in the same case already is adenomatous, examination of deeper levels may not be necessary, because it may not have any significant effect on the clinical management of the patient.
Conclusion

This study of the diagnostic utility of obtaining deeper levels in ND CR polyp specimens is, to our knowledge, the largest of its kind. We found an 8.0% increase in adenoma detection in 2 different laboratories by obtaining deeper levels on ND CR polyp biopsy specimens. The consistency of this number between our laboratories and compared with other smaller studies lends even more credulity to our findings. Owing to the potential impact of increased detection of adenomas, we recommend obtaining deeper-level sections on ND CR polyp biopsy specimens in an attempt to identify all adenomatous polyps. On the basis of the conclusions from this study and the high variability of rates from similar studies, laboratories should assess their own diagnostic conversion and adenoma detection rate from examination of deeper levels. We are currently undertaking a follow-up study to determine if the original policies that were implemented as a result of this study have reduced the number of ND CR polyp biopsy specimens in our practice.

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References