Quantitative Assessment of the Subepithelial Collagen Band Does Not Increase the Accuracy of Diagnosis of Collagenous Colitis

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Abstract

The thickness of eosinophilic band in collagenous colitis (CC) was assessed by 3 methods: histologic estimates (22 observers), conventional measurements using a calibrated micrometric scale (1 observer), and semiautomatic micrometric measurements (1 observer). By the histologic estimate technique, 7.4% of the results failed to diagnose CC; by calibrated micrometry, the failure was 6% and by semiautomatic micrometry, 6%. The main difficulty in measuring the thickness of the CC band is that the deeper border of the band appears fuzzy and hairy-irregular. CC should be defined not exclusively on the basis of the thickness of the collagen table, but as a microscopic constellation characterized by a distorted superficial cell arrangement, with areas of epithelial denudation and inflammatory cells in the superficial epithelium and the lamina propria. In agreement with Lazenby’s statement: “Focusing solely on the collagen band can result in both over- and underdiagnosis.”

In 1976, the Swedish pathologist Lindström1 described, in a patient with chronic watery diarrhea, the presence of a thick collagenous band underneath the luminal epithelium of the colonic mucosa.1 He called that disease collagenous colitis (CC). In the same year, Freeman et al2 described a similar association in 2 patients. Since these 2 publications included only 3 cases and the fourth case was reported 4 years later,3 collagenous colitis (CC) was considered a rare disease in the early 1980s. Even as recently as 1990, CC was regarded as “a relatively rare disorder.”4

In time, additional cases of CC were detected, and today, CC is considered a common disease, particularly at hospitals treating a large number of patients with gastrointestinal disorders. The estimated incidence of CC in different reports varies from 0.6 to 2.3 cases per 100,000 inhabitants5-7; a prevalence of 15.7 cases per 100,000 inhabitants has been reported.7

The diagnosis of CC is based on the microscopic demonstration of a thick hyaline (amorphous) eosinophilic band underneath the surface epithelium of the colon and, in some cases, of the rectum. Despite the fact that the thickness of the subepithelial amorphous band is the most important single parameter for the diagnosis of CC, there is no consensus among pathologists as to how thick the CC band should be. The various thicknesses of the collagenous band required for a diagnosis of CC include the following: 7 µm or more8-13; 10 µm or more14-16; thicker,17 exceeding18 or at least19 10 µm; 10 to 15 µm20; 12 µm or more21; and more than 15 µm.22 Other authors postulated that “any abnormal increase in the subepithelial collagen in the proper inflammatory background should be considered as diagnostic of collagen
Materials and Methods

Histologic Estimate

Filed, well-oriented H&E-stained sections from 22 consecutive patients having CC were photographed at ×40 magnification. Single prints of the 22 cases were used to question 16 senior and 6 junior pathologists. They were requested to calculate in micrometers the subepithelial band by comparing its thickness with another structure appearing in the same print, ie, the nucleus of a mature lymphocyte. Previous measurements with a calibrated ocular demonstrated that the nuclei in 93% of 1,000 consecutive mature lymphocytes were 5 µm in dimension (C.A.R. and Y. Kock, unpublished data, 1989). The eosinophilic table was estimated by guessing how many mature lymphocytes would be required to cover the thickness of the band. The 22 pathologists were briefed that the size given would be regarded as a “diagnostic commitment” and that deviations of ± 2 µm from the actual measurements on the prints (checked with a calibrated micrometric scale) would be regarded as a “diagnostic error.” The rationale for that “commitment” was that, according to current definition, a difference of ± 2 µm would be crucial because a band with a thickness of 9 µm would give no indication of CC, whereas a band of 11 µm would suggest CC. Senior and junior pathologists were asked to disregard other histologic features appearing in the prints.

In reviewing the data before analysis, it was noted that 2 different pairs of observers gave identical values for each sample, a situation very unlikely to occur even for the sole effect of chance; we therefore excluded from the analysis the data for 1 observer from each of these 2 pairs. Owing to the great number of missing values, we also decided to exclude 2 samples with 6 and 5 missing values of 14 overall missing in the complete data set; for the 3 remaining missing values, we assumed the mean value of their respective sample, rounded to the nearest integer value. After making these changes, our data set consisted of 19 observers and 20 samples, for a total of 380 measurements.

To graphically evaluate the interobserver concordance, a mean value was obtained for each reading by the 19 pathologists. These percentage deviations from mean sample value were then graphed as box plots (1 for each pathologist). The central body of box plots includes the values between the first and third quartiles, thus covering 50% of the values; vertical lines in both directions reach values up to 1.5 times the height of the central body, and the few external points exceed this rule.

Conventional Measurements Using a Calibrated Micrometric Scale

Filed colonoscopic sections from 87 consecutive patients having a previous histologic diagnosis of CC were retrieved. By means of a conventional ocular calibrated microscale, 1 senior pathologist (G.N.) measured the thickest fraction of the eosinophilic table at 3 different colonoscopic levels.

Semiautomatic Micrometric Measurements

 Archived colonoscopic sections from 34 consecutive patients having a histopathologic diagnosis of CC were retrieved. With a Soft Imaging System (Cell B) (Olympus Altra 20, Olympus, Tokyo, Japan), 1 senior pathologist (F.P.) measured the thickest fraction of the collagen table by moving the vector between the nearest luminal border and the deepest aspect of the band. The areas to be measured were labeled, and a second blinded measurement of each labeled area was carried out 1 week later.

Results

Histologic Estimates

Results of the calculated thickness are shown in Table 1. Table 1 shows that in the majority of the prints, the band was 17 µm thick or thicker, as assessed with the simultaneously enlarged calibrated microscale. Of 380 measurements, 92.6% (352/380) were 10 µm or more, a value indicative of CC, and in the remaining 28 cases (7.4%), the band thickness was estimated to be 9 µm or thinner, a measurement not indicative of CC.

One patient having watery diarrhea and other histologic features of CC, such as partial denudation of the surface mucosa and inflammatory cells, had the thinnest CC band of the prints, namely 10 µm (assessed with the simultaneously enlarged calibrated microscale; see the “Materials and Methods” section). With regard to that particular print, 18 of 19 observers estimated that the band measured 9 µm or smaller, a thickness not compatible with CC.

The other 19 samples with a much thicker CC band were considered by the great majority of observers (at least 16 of 19) as compatible with CC because the thickness was estimated to be 10 µm or more. However, some pathologists gave disparate values of the thickness of the band (eg, values for sample 7 ranged from 16 to 100 µm) (Table 1).
The box plots in Figure 1 show that for 6 of 19 pathologists, the central body was below the zero value, denoting an overall tendency to underestimate the value of the CC band (for 2 pathologists, the values attributed to all samples were below the respective mean value). On the other hand, the central body of box plots was above the zero value for 4 observers, and no pathologist assigned to all samples a value exceeding the respective average values.

Conventional Measurements Using a Calibrated Micrometric Scale

When the thickest portion of the CC band was assessed at 3 colonic levels by means of conventional micrometry, it was found that the band measured 10 µm or more (compatible with CC) in 82 (94%) of 87 cases. Nevertheless, a wide variation in the thickness of the band was sometimes recorded for the 3 colonic sites, and in 8 of 82 cases, 1 or

![Box plots of the percentage of deviation from the mean sample value for 19 pathologists (using the “eyeball” method) and 20 samples. The central body of each box plot includes the values between the first and third quartiles, thus covering 50% of the values; vertical lines in both directions reach values up to 1.5 times the height of the central body, and the few external points exceed this rule.](image-url)
2 measurements gave a value less than 10 \( \mu \text{m} \) \textbf{Table 2I}. In the remaining 5 cases (6%), the thickest band was found to be 9 \( \mu \text{m} \) or smaller, a value not consistent with the diagnosis of CC.

\section*{Semiautomatic Micrometric Measurements}

The results in \textbf{Table 3I} show that the thickness of the band assessed by this method was 10 \( \mu \text{m} \) or more (compatible with CC) in the first and second measurements for 31 (91%) of 34 cases. However, a substantial discrepancy in estimating the thickness was frequently recorded between the 2 consecutive measurements when using this method, with a maximum difference of more than 50 \( \mu \text{m} \). In 1 of the remaining 3 cases (3%), the first measurement was 9.1 \( \mu \text{m} \) (not compatible with CC), whereas the second measurement of the same area was 11.6 \( \mu \text{m} \) (compatible with CC), with a mean value of 10.3 \( \mu \text{m} \). Finally, for 2 cases (6%), both measurements gave a result of less than 10 \( \mu \text{m} \), a value not consistent with the diagnosis of CC.

\section*{Discussion}

In this retrospective survey of colonic sections from patients with a previous clinicopathologic diagnosis of CC, the thickness of the CC band was assessed by 3 methods. When the thickness of the eosinophilic colonic band was estimated by the “eyeball” technique, 7.4% of the results

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Sample No. & Site 1 & Site 2 & Site 3 \\
\hline
1 & 14 & 12 & 15 \\
2 & 10 & 8 & 7 \\
3 & 12 & 9 & 8 \\
4 & 9 & 8 & 10 \\
5 & 17 & 16 & 20 \\
6 & 20 & 19 & 22 \\
7 & 6 & 7 & 6 \\
8 & 10 & 8 & 10 \\
9 & 15 & 12 & 12 \\
10 & 9 & 7 & 6 \\
11 & 12 & 10 & 8 \\
12 & 20 & 14 & 15 \\
13 & 14 & 16 & 12 \\
14 & 28 & 24 & 22 \\
15 & 12 & 14 & 14 \\
16 & 17 & 15 & 18 \\
17 & 14 & 12 & 12 \\
18 & 24 & 18 & 15 \\
19 & 15 & 16 & 12 \\
20 & 38 & 36 & 32 \\
21 & 30 & 27 & 25 \\
22 & 16 & 15 & 12 \\
23 & 15 & 16 & 14 \\
24 & 18 & 17 & 17 \\
25 & 22 & 20 & 18 \\
26 & 20 & 16 & 17 \\
27 & 14 & 12 & 14 \\
28 & 9 & 7 & 7 \\
29 & 24 & 26 & 24 \\
30 & 28 & 25 & 27 \\
31 & 12 & 10 & 10 \\
32 & 25 & 23 & 20 \\
33 & 16 & 15 & 15 \\
34 & 13 & 12 & 12 \\
35 & 9 & 6 & 6 \\
36 & 28 & 22 & 24 \\
37 & 27 & 21 & 20 \\
38 & 14 & 12 & 9 \\
39 & 21 & 15 & 12 \\
40 & 6 & 6 & 5 \\
41 & 12 & 10 & 10 \\
42 & 20 & 16 & 16 \\
43 & 13 & 7 & 8 \\
44 & 15 & 14 & 10 \\
\hline
\end{tabular}
\caption{Collagenous Colitis Band Thickness of 87 Samples Measured at Three Colonic Sites With a Conventional Calibrated Micrometric Scale}
\end{table}
failed to diagnose CC. With calibrated micrometry, the failure to “diagnose” CC was 6%, and with semiautomatic micrometry, it was 6%. Failure to diagnose CC by the histologic estimate method did not depend on the experience of the observer (senior vs junior pathologist) or the gender of the pathologist (data not shown).

The results obtained suggest that, in assessing the thickness of the CC band, conventional micrometry and the Soft Imaging System (Cell B) are more effective than the histologic estimate method, even if the difference in performance is minimal. However, the correct diagnoses by the 3 methods, specifically, 92.6%, 94%, and 94%, were established on prints or sections having an obviously broad CC band; some authors claim that it should be 7 µm, and others indicate 0.4 to 3.65 µm, or more, of the normal basement membrane of the colonic mucosa (BM); some authors state that the BM should measure 0 to 3 µm, and others indicate 4.0 to 2.3 µm, 2.5 µm, 3.65 µm, 4 µm, 4.6 to 6.9 µm, 6 µm or thinner, 12 µm or thinner, or even 15 µm. Likewise it should be noted that there is no consensus regarding the actual thickness of the BM; some authors state that the BM should measure 0 to 3 µm, and others indicate 4.0 to 2.3 µm, 2.5 µm, 3.65 µm, 4 µm, 4.6 to 6.9 µm, 6 µm or thinner, 12 µm or thinner, or even thinner than 15 µm.

The reason for the variation in size of the CC band (and of the BM) in the different series remains puzzling. The possibility that the size of the CC band (and of the BM) differs in disparate geographic regions could be one of the explanations. In some countries, patients with CC consult when early symptoms ensue, whereas in other countries, patients consult for more advanced, chronic symptoms. However, this possibility would seem unlikely because the thickness of CC band has been found to be unrelated to the duration of clinical symptoms.

The main difficulty in measuring the thickness of the CC band is that the deeper border of the table is not easy to delineate by light microscopy because it appears fuzzy, shaggy, and hairy-irregular, a phenomenon pointed out by Lazenby. To better visualize the collagenous band in CC, Lazenby et al stained sections with Masson-trichrome stain. Following this recommendation, we stained sections of cases of CC with a trichrome stain. Image 1 shows that the deeper border of the table appears somewhat irregular in Masson-trichrome stain. After processing a duplicate (ie, a “cloning”) of Image 1 with the INVERT function of a Photoshop program (Ps Adobe Photoshop CS3 extended, Adobe, San Jose, CA), the irregularities and “fuzziness” of the lower border of the collagen band were substantially highlighted Image 2.

### Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>First Measurement</th>
<th>Second Measurement</th>
<th>Difference (Second – First)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45.0</td>
<td>54.0</td>
<td>9.0</td>
</tr>
<tr>
<td>2</td>
<td>26.8</td>
<td>31.6</td>
<td>4.8</td>
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<td>3</td>
<td>16.1</td>
<td>13.3</td>
<td>–2.8</td>
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<tr>
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<td>21.6</td>
<td>54.1</td>
<td>32.5</td>
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<td>5</td>
<td>10.8</td>
<td>26.8</td>
<td>16.0</td>
</tr>
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<td>6</td>
<td>73.4</td>
<td>21.8</td>
<td>–51.6</td>
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<td>7</td>
<td>74.8</td>
<td>15.5</td>
<td>–59.3</td>
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<td>10.3</td>
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<tr>
<td>9</td>
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<td>40.9</td>
<td>3.7</td>
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<td>9.1</td>
<td>11.6</td>
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<td>45.2</td>
<td>19.3</td>
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<td>39.5</td>
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<td>87.6</td>
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<td>18.0</td>
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<td>60.1</td>
<td>16.6</td>
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<td>36.7</td>
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<td>32.0</td>
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<td>9.0</td>
<td>0.4</td>
</tr>
<tr>
<td>34</td>
<td>5.1</td>
<td>5.3</td>
<td>0.2</td>
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</table>

* Measurements are in micrometers.
In previous studies, a novel method was described to investigate the thickness of the collagen band by observing H&E-stained sections with incident-light fluorescence using an Axioscope, Leitz, fluorescent microscope and green H filter at 546-nm wave length. By this method, it was also found that the eosinophilic band in CC was usually fuzzy and hairy-irregular. These limitations might be the main confounding factor for the discrepancies found not only in the present work but also in the literature.

Today, many pathologists rely too heavily on the quantitative measure of the subepithelial collagen band to make a diagnosis of CC. But the increased thickness of the band is associated with other histologic parameters of CC, as pointed out by Lazenby et al. years ago. In fact, the superficial epithelium shows flat or cuboidal cells; pseudostratification with hyperchromatic, irregular nuclei and eosinophilic cytoplasm; goblet cell depletion; and mucin-free vacuoles. There is also slight intraepithelial lymphocytosis, occasional neutrophilic and eosinophilic granulocytes, and apoptotic granules. In addition, the superficial lamina propria contains small to moderate numbers of lymphocytes, plasma cells, mast cells, granulocytes, and eosinophils. Thus, a series of cellular anomalies and inflammatory changes are associated with the broad eosinophilic band in the histologic diagnosis of CC.

None of the three methods applied in this survey gave fully reliable information regarding the exact vertical thickness of the eosinophilic table of CC. Perhaps CC should not be defined exclusively on the basis of the thickness of a broad collagen table, but as a microscopic constellation characterized by a distorted superficial cell arrangement, with areas of epithelial denudation and inflammatory cells in the superficial epithelium and the lamina propria, in addition to an abnormally thick subepithelial, continuous, eosinophilic band. Hence, the sum of these histologic parameters should be part of a microscopic definition of collagenous colitis (CC). In agreement with Lazenby’s statement: “Focusing solely on the collagen band can result in both over- and underdiagnosis.”

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