

## Endoscopic biopsy technique in the diagnosis of celiac disease: One bite or two?

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**Background:** The diagnosis of celiac disease is dependent on the quality of biopsy specimens obtained at EGD. Endoscopists may obtain a single- or double-biopsy specimen with each pass of the forceps.

**Objective:** To compare the quality of biopsy specimens obtained with the single-biopsy and double-biopsy techniques.

**Design:** Prospective cohort study.

**Setting:** U.S. tertiary-care university hospital.

**Patients:** Patients undergoing upper endoscopy with confirmed, suspected, or unknown celiac disease status.

**Interventions:** Four biopsy specimens from the second portion of the duodenum: 2 by using the single-biopsy technique (1 bite per pass of the forceps) and an additional 2 by using the double-biopsy technique (2 bites per pass of the forceps). Specimens were blindly reviewed to determine orientation, consecutive crypt-to-villous units, and Marsh score.

**Main Outcome Measurements:** Proportion of well-oriented biopsy specimens.

**Results:** Patients (N = 86) were enrolled, 47% with known celiac disease, 36% with suspected celiac disease, and 17% with an unknown celiac disease status. Well-oriented biopsy specimens were noted in 66% of patients with the single-biopsy technique and 42% of patients with the double-biopsy technique ( $P < .01$ ). Analysis of matched pairs showed improved orientation with the single-biopsy technique (odds ratio 3.1; 95% confidence interval, 1.5-7.1;  $P < .01$ ). This persisted in subgroup analysis of patients with known celiac disease ( $P = .02$ ), villous atrophy ( $P = .02$ ), and a final diagnosis of celiac disease ( $P < .01$ ).

**Limitations:** A single-center trial.

**Conclusion:** The single-biopsy technique improves the yield of well-oriented duodenal biopsy specimens. Endoscopists should consider taking only 1 biopsy specimen per pass of the forceps in patients undergoing biopsies of the duodenal mucosa. (*Gastrointest Endosc* 2015;81:1228-33.)

Celiac disease is a multisystem autoimmune disease that is triggered in genetically predisposed individuals by the ingestion of gluten-containing products. It affects

approximately 1% of the population worldwide, although the disease in a large proportion of patients remains undiagnosed.<sup>1</sup> In the United States, a recent analysis of

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the National Health and Nutrition Examination Survey data revealed that celiac disease is diagnosed in only 17% of individuals currently.<sup>2</sup> The criterion standard for diagnosing celiac disease is a biopsy specimen obtained from the proximal small intestine that shows villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis in a patient with clinical features of gluten sensitivity.<sup>3</sup>

Guidelines recommend that 4 to 6 separate biopsy specimens be obtained from the proximal duodenum when celiac disease is suspected.<sup>1,4,5</sup> This is due in part to the patchy nature of the disease but also to the low yield of adequately oriented biopsy specimens.<sup>6-8</sup> A well-oriented biopsy specimen is defined as a piece of intestinal mucosa that displays 4 consecutive, parallel, crypt-to-villous units that are visualized along their entire lengths. Previous studies have found poor rates of adequately oriented biopsy specimens, accounting for as many as 30% of the specimens.<sup>7</sup> This observed low yield of oriented specimens greatly limits the diagnosis and management of celiac disease.<sup>9</sup>

Despite the existence of established guidelines, less than half of all patients undergoing endoscopy for symptoms suggestive of celiac disease undergo the recommended 4 to 6 biopsies. In fact, most patients undergo only a total of 2 biopsies.<sup>10</sup> One reason why fewer biopsy specimens are taken is that the acquisition of individual specimens increases procedural time. To minimize procedural time, endoscopists often obtain 2 biopsy specimens from a single pass of the biopsy forceps (2 bites, double-biopsy technique). Another method is to obtain 1 biopsy specimen per pass of the biopsy forceps (1 bite, single-biopsy technique). In actual clinical practice, the choice is made according to the clinician's personal preference. However, the effect of these 2 techniques on the quality of small intestine biopsy specimens is unknown.

Overall, only a few studies have evaluated the effect of biopsy technique on the quality of histopathology.<sup>11</sup> Some have compared capsule and endoscopic techniques without significant difference.<sup>12-14</sup> Others have compared different forceps and have shown varying results.<sup>15-19</sup> Results of previous studies comparing the single- and double-biopsy techniques throughout different areas of the GI system are also conflicting. One study conducted in patients with ulcerative colitis evaluated the quality of colonic biopsy specimens obtained with 1- and 2-bite biopsies and found that the single-biopsy technique produced superior quality specimens.<sup>20</sup> Other studies conducted in the proximal GI tract found no difference in the quality of specimens obtained from single and double biopsies.<sup>17-19</sup>

The primary aim of this study was to compare the number of well-oriented duodenal biopsy specimens obtained with the single- and double-biopsy techniques. The secondary aim was to compare differences in Marsh score with these 2 methods among patients with suspected or confirmed celiac disease.

## METHODS AND MATERIALS

### Patients

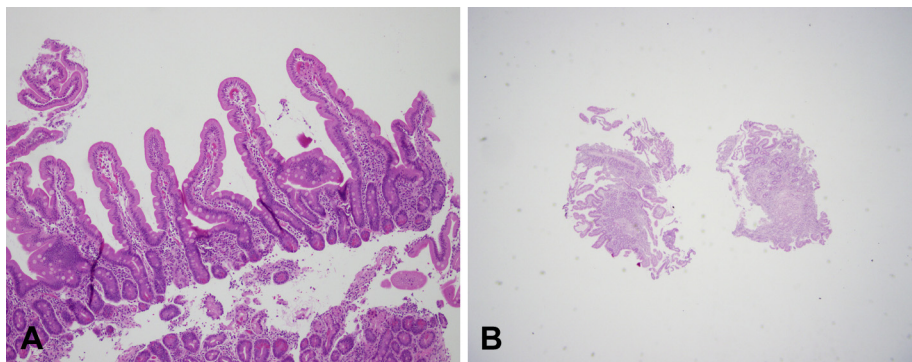
From August 2012 to February 2013, we prospectively evaluated patients undergoing EGD at Columbia University Medical Center. The study included patients with known celiac disease, suspected celiac disease (based on serology, family history, or symptoms), and unknown celiac disease status but who underwent EGD for indications unrelated to celiac disease. Only patients 18 years of age or older were enrolled in the study. Informed consent was obtained from all patients. Three gastroenterologists performed the endoscopies. The Columbia University Institutional Review Board approved this study (AAAJ8855). No funding was needed for this study.

### Procedure

Each patient underwent a standard EGD that included a total of 4 biopsy specimens taken from the second portion of the duodenum. Biopsy specimens were also taken from other portions of the GI tract, including the duodenal bulb, when clinically indicated. Only specimens obtained from the second portion of the duodenum were evaluated in this study. All specimens were obtained by using Boston Scientific Single-Use Radial Jaw 4 (Boston Scientific, Natick Mass) (large capacity with needle, 2.8-mm working channel (catalog reference # 1333)). Two specimens were first obtained by using the double-biopsy technique (2 bites) and placed in a container. An additional 2 specimens were then obtained by using the single-biopsy technique (1 bite) and placed in a separate container. No attempts were made to orient the biopsy samples in the endoscopy suite or after fixation in 10% neutral buffered formalin, as is routine practice at our institution. All biopsy specimens were processed according to standard histologic protocols and stained with hematoxylin and eosin.

### Study design

An experienced GI pathologist, blinded to indication and biopsy technique, reviewed all specimens to determine the adequacy of orientation and the total number of biopsy specimens obtained and to assign a modified Marsh score.<sup>18</sup> Biopsy specimens were considered adequate for evaluation of Marsh score if they manifested 4 consecutive crypt-villous units (Fig. 1A). Biopsy specimens with fewer than 4 consecutive crypt-to-villous units were considered to be of poor quality for evaluation of Marsh scores (Fig. 1B). Specimens were then evaluated for villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis. A modified Marsh score was assigned based on the presence and severity of these features. A diagnosis of celiac disease was made in patients with suspected celiac disease who were found to have villous atrophy and intraepithelial lymphocytosis on biopsy (defined as a Marsh score of 3A or higher). A subset of randomly selected



**Figure 1.** **A**, A representative image of a well-oriented duodenal biopsy specimen with 6 consecutive, normal-appearing, crypt-to-villous units (H&E, orig. mag.  $\times 10$ ). **B**, A representative image of a poorly oriented duodenal biopsy specimen with no consecutive crypt-to-villous units (H&E, orig. mag.  $\times 2$ ).

specimens were evaluated by a second GI pathologist to determine orientation. This pathologist was also blinded to indication and biopsy technique.

Differences in outcomes between the single- and double-biopsy technique were analyzed among primary and secondary subgroups. Primary subgroups divided patients based on their initial celiac history at the time of EGD (known, suspected, or unknown). Secondary subgroups (final diagnosis of celiac disease and villous atrophy) were established by incorporating outcomes of histopathology. The subgroup of patients with a final diagnosis of celiac disease included patients with known disease and those with suspected disease who were found to have villous atrophy on biopsy. The subgroup of patients with villous atrophy incorporated patients from all primary subgroups who demonstrated evidence of villous atrophy on biopsy. Finally, differences in Marsh score were compared for all patients between the single- and double-biopsy techniques.

### Statistical analysis

The study was powered for the primary outcome of specimen orientation using the McNemar test for matched pairs. To perform a power calculation, we assumed that 35% of paired samples would be discordant. At the  $\alpha = .05$  level of confidence, a sample size of 85 pairs yielded 80% power to detect a minimum 19% improvement in orientation by using the single-biopsy technique compared with the double-biopsy technique. Categorical variables were analyzed using  $\chi^2$  test for data satisfying the central limit theorem and *t* tests for continuous data. For continuous variables, summary data were examined graphically, and medians and means were calculated. All data were analyzed by using SAS version 9.3 (SAS Institute, Cary, NC).

## RESULTS

A total of 86 patients were enrolled in the study. Their characteristics are shown in Table 1. Overall, 57 patients

**TABLE 1. Characteristics of patients (N = 86)**

Sex, no. (%)	
Female	59 (69)
Male	27 (31)
Age, y, mean $\pm$ SD	
	49 $\pm$ 18.9
Indication for EGD, no (%)	
Follow-up CD	40 (47)
Suspected CD	31 (36)
Unknown CD	15 (17)

CD, Celiac disease.

(66%) had well-oriented biopsy specimens with the single-biopsy technique and 36 patients (42%) had well-oriented biopsy specimens with the double-biopsy technique ( $P < .01$ ). Improved orientation with the single-biopsy technique was also observed on analyzing matched pairs (odds ratio 3.1; 95% confidence interval, 1.5-7.1,  $P < .01$ ). Biopsy specimens from 50 randomly selected patients were analyzed by a second GI pathologist. The trend of improved orientation with the single-biopsy technique persisted on analysis by the second pathologist (odds ratio 7.0; 95% confidence interval, 2.1-36.7,  $P < .01$ ).

A mean of 2.4 biopsy specimens per patient were obtained with the single-biopsy technique, and 2.0 biopsy specimens per patient were obtained with the double-biopsy technique ( $P < .05$ ). Two biopsy specimens were successfully obtained from 60 patients (70%) with the single-biopsy technique and from 49 patients (57%) with the double-biopsy technique. No patients were noted to lose both specimens. There was a significant loss of 1 specimen noted in only 2 patients (2%) with the single-biopsy technique and in 19 patients (22%) with the double-biopsy technique ( $P < .01$ ). The remainder of patients had more than 2 biopsy specimens: 24 patients (28%)

**TABLE 2. Comparison of outcomes with single- and double-biopsy techniques**

	Single-biopsy technique	Double-biopsy technique	P value
<b>Biopsy features</b>			
Patients with oriented specimens, no. (%)	57 (66)	36 (42)	<.01
Mean consecutive crypt-to-villous units	4.93	3.92	<.05
Mean no. of biopsy specimens per patient	2.4	2.0	.05
<b>Patients with oriented specimens by subgroup, no. (%)</b>			
Known CD (n = 40)	25 (63)	14 (35)	.02
Suspected CD (n = 31)	23 (74)	17 (55)	.21
Unknown CD (n = 15)	9 (60)	5 (33)	.22
Villous atrophy (n = 26)	22 (85)	12 (46)	.02
Final diagnosis of CD (n = 52)	35 (67)	19 (37)	.01
<b>Final Marsh score by orientation (N = 86), no. (%)</b>			
Normal/ nonspecific	48 (56)	57 (66)	.18
Marsh 1	15 (17)	7 (8)	
Marsh 2	—	—	
Marsh 3A	8 (9)	9 (10)	
Marsh 3B	11 (13)	7 (8)	
Marsh 3C	4 (5)	6 (7)	

CD, Celiac disease.

with the single-biopsy technique and 18 patients (21%) with the double-biopsy technique.

The average number of well-oriented consecutive crypt-to-villous units was 4.93 (median 4, range 0-20) with the single-biopsy technique and 3.92 (median 3, range 0-21) with the double-biopsy technique ( $P < .05$ ) (Table 2).

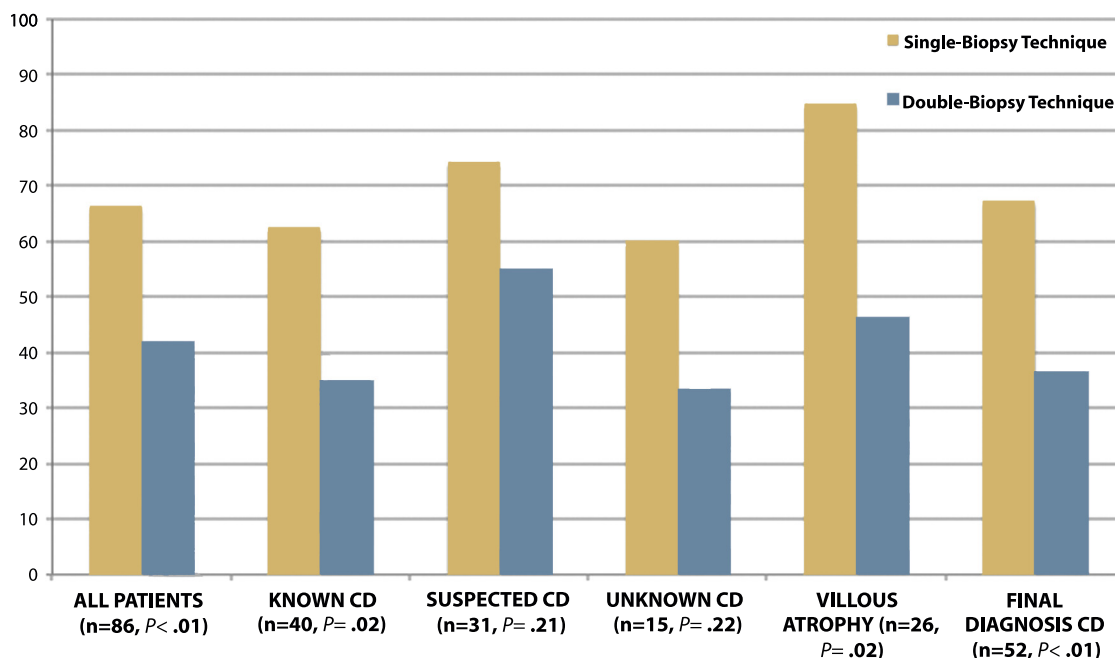
Villous atrophy was identified in 26 patients (33%) and led to a new diagnosis of celiac disease in 12 patients (39%) who underwent EGD for suspected celiac disease. This resulted in a final diagnosis of celiac disease in 52 patients. When matched pairs were assessed in subgroups, there was improved biopsy orientation with the single-biopsy technique in patients with known celiac disease ( $n = 40$ ,  $P = 0.02$ ), villous atrophy ( $n = 26$ ,  $P = .02$ ) and a final diagnosis of celiac disease ( $n = 52$ ,  $P < .01$ ) (Table 2, Fig. 2).

There was concordance of Marsh scores between the single- and double-biopsy techniques in 57 patients (66%). Discordant Marsh scores were observed in 29 patients: 18 patients (21%) in whom greater severity according to the Marsh score was noted with the single-biopsy technique and 11 patients (13%) in whom greater severity according to the Marsh score was noted with the double-biopsy technique ( $P = .18$ ). In 13 of 18 patients, the single-biopsy technique captured new features

supporting a diagnosis of celiac disease (increased intraepithelial lymphocytes and/or villous atrophy) not seen in specimens from the double-biopsy technique. In 5 of 11 patients, the double-biopsy technique captured new features supporting a diagnosis of celiac disease (increased intraepithelial lymphocytes and/or villous atrophy) not seen in specimens from the single-biopsy technique.

## DISCUSSION

The results of our study demonstrate improved orientation of biopsy specimens with the single-biopsy technique compared with the double-biopsy technique. Statistical significance persisted on subgroup analysis of patients with known celiac disease, villous atrophy, and a final diagnosis of celiac disease. Given the dependence of diagnosis on mucosal architecture, obtaining well-oriented specimens with continuous segments of crypt-to-villous units is of critical importance in celiac disease. We also observed a trend toward increased severity of disease in specimens obtained with the single-biopsy technique; however, our study was not powered for this outcome and did not achieve significance. It is interesting to note that in certain cases, intraepithelial lymphocytosis and/or villous atrophy was only



**Figure 2.** Percentage of well-oriented specimens by technique and subgroup. *CD*, celiac disease.

identified in specimens taken with the single-biopsy technique. This is of clinical importance given that some patients with celiac disease may initially manifest with only intraepithelial lymphocytosis.<sup>21</sup> We would argue that taking 1 biopsy per pass of the forceps does not mean that the recommended number of samples be reduced, but that practitioners consider using the single-biopsy technique to obtain the 4 to 6 specimens necessary for the assessment of celiac disease.

Our study showed that specimens obtained with the double-biopsy technique were at increased risk of damage and procedural loss. There were significantly fewer crypt-to-villous units noted on specimens obtained with double biopsies, thus suggesting smaller pieces and architectural damage. There was also a significant difference in the incidence of specimen loss between the 2 techniques with the double-biopsy technique showing worse outcomes. We had intended to use the mean number of pieces per technique as a surrogate for loss and fragmentation, but this value is difficult to interpret as it may also represent pieces that were obtained across mucosal folds. One way to account for our findings is the needle at the center of the biopsy forceps. Although the purpose of this feature is to facilitate the retention of multiple specimens, it likely contributes to fragmentation and limited accommodation within the cusps of the forceps when a second specimen is introduced. Despite the observed disadvantages of the double-biopsy technique, it persists in clinical practice due to its ability to minimize procedural time.<sup>10</sup> Going forward, focus should be placed on optimizing mucosal sampling in celiac disease by using the single-biopsy technique.

It is important to highlight other factors that may have affected the quality of the biopsy material in our study. First, we did not randomize our sampling of single and double biopsies. Second, there continues to be debate regarding the handling and processing of biopsy specimens after they are obtained. Some suggest that orienting biopsy specimens in the endoscopy unit before fixation helps to improve the quality of specimens.<sup>22</sup> However, no studies have ever evaluated the efficacy of this step in clinical practice as it relates to celiac disease. In North America, specimens are typically not oriented in the endoscopy suite before fixation.<sup>5,23</sup> This is often considered a time-consuming step that requires training and expertise of endoscopy assistants. In our study, we did not orient specimens before fixation, which reflects standard practice. Additionally, technicians in most North American pathology laboratories do not attempt to orient specimens after fixation or before processing and embedding biopsy specimens in paraffin tissue blocks.

We also recognize factors that may have limited our diagnosis of celiac disease. Current medical literature suggests improved diagnostic yield in patients with known and suspected celiac disease when duodenal bulb specimens are included in evaluation.<sup>24</sup> Although we recognize the utility of duodenal bulb biopsy in clinical practice, this was excluded from our analysis to limit confounders.

In summary, our study has demonstrated the superiority of the single-biopsy technique in the assessment of the duodenal mucosa for celiac disease, as indicated by its ability to produce a greater number of samples and a



greater proportion of well-oriented specimens. With this information, we must begin to question the utility of the 2 bite or double-biopsy technique in general clinical practice, as it has been shown to be inferior to the 1 bite or single-biopsy technique in both celiac disease and ulcerative colitis. The single-biopsy technique should also be formally evaluated in other disease processes that are dependent on a histopathology diagnosis, such as Barrett's esophagus. Similar to celiac disease, Barrett's esophagus is also characterized by a patchy distribution.

Given the inherently low yield of well-oriented specimens in the evaluation of celiac disease, it is important for endoscopists to consider taking only 1 biopsy specimen per pass of the forceps in patients undergoing biopsies of duodenal mucosa.

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