

Short Communication

Disposable *versus* Reusable Biopsy Forceps for Colorectal Epithelial Cell Proliferation in Humans¹

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Abstract

The performance of various measures of rectal mucosal proliferation has been evaluated in the literature, but the performance of the forceps used to obtain the tissue has received little attention. We used data from two large studies of proliferation at a single institution to compare reusable and disposable endoscopic forceps. Endoscopic pinch biopsies were taken 10 cm from the anal verge using either reusable or disposable, oval-cupped, sheathed forceps. The specimens were fixed, embedded, and sectioned, taking care to orient the specimens longitudinally. Five sections were placed on each slide. We determined how many slides did not contain eight scorable crypts (inadequate) and how many sections were necessary to identify eight complete crypts. There were 395 subjects who had biopsies taken with reusable forceps and 185 subjects who had biopsies taken with disposable forceps. The specimens were inadequate in 27.6% of the reusable forceps specimens *versus* 2.7% of the disposable forceps ($P < 0.0001$). The mean number of tissue sections necessary to identify eight scorable crypts for the reusable forceps was 3.82 (SD, 0.87) compared with 3.17 (SD, 0.83) for disposable forceps ($P = 0.0001$). The specimens taken with the disposable forceps were better, probably because the forceps were sharper. We believe that the better quality of the specimens and the sterility justify the higher cost of disposable forceps. We would urge investigators in proliferation studies to evaluate the biopsy equipment as carefully as they evaluate other aspects of their methods.

Introduction

There is a growing interest in the use of surrogate end point biomarkers as measures of colorectal cancer risk and in chemoprevention trials (1). Measures of mucosal proliferation are among the most widely studied (2). The published literature on

this topic has paid considerable attention to various methods to measure proliferation and strategies for statistical analysis. Less attention has been given to techniques for obtaining tissue specimens. For the past 8 years, we have been conducting studies of rectal mucosal proliferation. The large number of specimens that we have obtained during this research provided us the opportunity to compare specimens obtained by reusable and disposable forceps. In this short communication, we compare the quality of specimens obtained with different forceps and provide some practical recommendations for proliferation studies.

Materials and Methods

The methods that we have used for these studies have been described in greater detail elsewhere (3–5). The purpose of the studies was to measure rectal mucosal proliferation by immunohistochemical staining for proliferating cell nuclear antigen one of the commonly used proliferative markers.

Briefly, we enrolled individuals who were scheduled for clinically indicated colonoscopy. Each subject underwent preparation using either a balanced electrolyte/polyethylene glycol lavage solution or a phosphate-containing purge. At the start of the endoscopic procedure, a biopsy forceps (either reusable or disposable) was passed through the operating channel of a standard colonoscope. Six biopsies were taken at a distance of 10 cm from the anal verge, carefully transferred to bibulous paper, and placed in Steinberg's modified Eagle's medium. The specimens were processed according to standard histological procedures. Five sections were placed on poly-L-lysine-coated slides (Sigma Chemical Co. St. Louis, MO) and were taken at least 50 μm apart so that each would contain different crypts. Slides were incubated for 12–20 h at 4°C using PC10 antibody (Dako Co., Carpinteria, CA) at 1:100 in PBS/BSA. Detection of proliferating cell nuclear antigen was performed using the BioGenex SterAvigen Super Sensitive kit for alkaline phosphatase (Biogenex, San Ramon, CA). The slides were counterstained with Mayers hematoxylin and mounted with Eukitt (Calibrated Instruments, Inc., Hawthorne, NY). For each crypt, the total number of cells in the crypt, the center cell, and the ordinal number of stained nuclei were recorded. Only deeply stained nuclei were counted.

Crypts were designated as scorable if they were well oriented, if the entire length was visible in the longitudinal section, and if the base of the crypt touched the muscularis mucosa. If the muscularis was missing, a crypt was acceptable if its height was uniform with other crypts. For each study subject, at least 8 and at most 12 crypts were scored from each of two biopsies. Tissue sections from each of the four biopsies were screened under low power, and the two slides that had the best orientation were selected for scoring (*i.e.*, had the highest number of potentially scorable crypts). Selection was based on orientation and not on the amount of staining. Beginning with the first scorable crypt in the first section, successive crypts and successive sections were counted, if necessary, until at least

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eight crypts had been scored. If there were fewer than eight scorable crypts in a biopsy, the biopsy was designated as inadequate, and the next biopsy was selected. If there were fewer than eight scorable crypts in the second biopsy, it was also designated inadequate, and additional blocks were cut and scored.

To compare the quality of specimens, we determined the average number of sections necessary to obtain eight scorable crypts. With a better quality specimen, it requires fewer sections to identify eight scorable crypts and there are fewer inadequate biopsies. We determined the mean number of sections reached at the eighth scorable crypt for each subject and determined the grand mean for each subject. We then compared group means using Student's *t* test, and we compared the subject means using the Wilcoxon rank sum test using the NPAR1WAY procedure in SAS (Cary, NC). To compare the proportion with inadequate biopsies we used Pearson's χ^2 test.

The reusable forceps were standard sized (not jumbo), sheathed, fenestrated, oval forceps that were passed through the operating channel of conventional colonoscopes. The forceps were not routinely sharpened. They were taken out of service when they malfunctioned. Disposable forceps were similar in design—sheathed, oval, and fenestrated—but were used only once. Both the reusable and the disposable forceps were obtained from different manufacturers during the course of the study.

Results

There were 395 subjects who had biopsies taken with reusable forceps and 185 subjects with biopsies taken with disposable forceps. The specimens were inadequate in 27.6% of the reusable forceps specimens *versus* 2.7% of the disposable forceps ($P < 0.0001$). The mean number of sections necessary to identify eight scorable crypts for the reusable forceps was 3.82 (SD, 0.87) compared with 3.17 (SD, 0.83) for disposable forceps ($P = 0.0001$). Using the nonparametric Wilcoxon test, the subject mean number of sections for the disposable forceps was lower than the reusable forceps ($P = 0.001$). Examples of biopsies taken with the disposable and reusable forceps are shown in Fig. 1. These photomicrographs serve as examples to demonstrate the appearance of adequate and inadequate biopsies; they are not necessarily representative.

Discussion

The equipment used to obtain tissue for rectal mucosal proliferation measures can have a significant impact on the quality of the specimens that are obtained. We have shown that disposable biopsy forceps obtain more reliable tissue samples than reusable forceps.

Several different techniques for obtaining rectal tissue have been evaluated. Bostick *et al.* (6) used rigid forceps passed through a rigid sigmoidoscope. This method obtains the largest samples, but because of the large size they may be difficult to orient. The deeper biopsies are more likely to result in bleeding complications. Visualization of the mucosa is better than with “blind” biopsies but inferior to the view with a flexible sigmoidoscope.

Biopsies can also be taken using “jumbo” forceps. These jumbo forceps do not fit through the biopsy channel of a standard diagnostic endoscope and require either a special therapeutic instrument, not available in all settings, or require affixing the forceps to a semi-rigid rod (usually a disposable rigid sigmoidoscopy swab). The specimens obtained with the jumbo

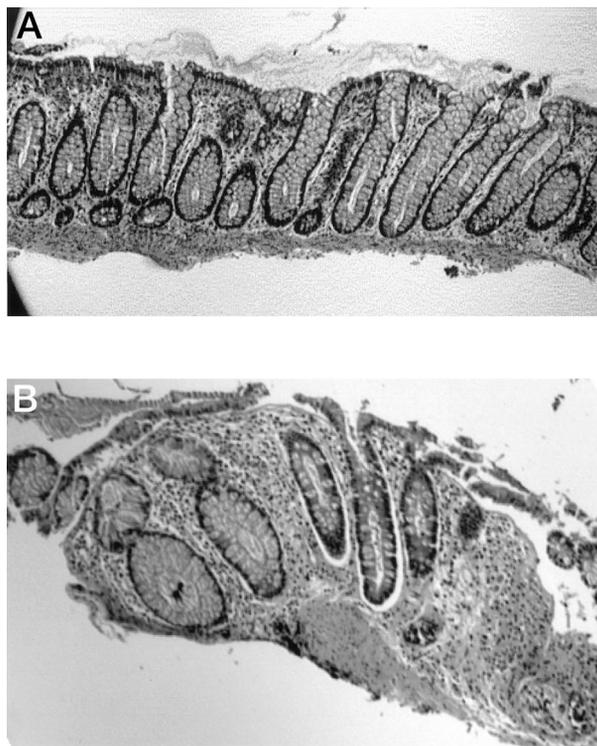


Fig. 1. A, example of an adequate biopsy taken using a disposable forceps. H&E, $\times 4$. B, example of an inadequate biopsy taken using a reusable forceps. H&E, $\times 4$.

forceps are larger in size than the standard endoscopy forceps but are also associated with slightly higher risk of bleeding.

In our studies, we use standard size biopsy forceps that are passed through the operating channel of conventional endoscopes. These forceps have an unparalleled record of safety. We have taken pinch biopsies from over 1000 patients using this technique and never had a bleeding complication. We believe that safety is extremely important in these studies of volunteers. We also believe that carefully examining the distal bowel with a flexible endoscope provides useful information about polyp status that is not possible with blind biopsies or biopsies taken through rigid instruments.

In this report, we document the greater yield from disposable forceps. This is most likely attributable to the fact that the disposable forceps are, on average, sharper than the reusable forceps. Reusable forceps can become dull with repeated use and mechanical cleaning. In addition, the hinge and cable mechanism of the disposable forceps are likely to operate more smoothly and reliably and are less likely to malfunction because they are used only once.

There is another advantage to disposable forceps, sterility. There is a very small but real risk of transmitting infection with reusable equipment, despite the most zealous efforts at disinfection. The added margin of safety, although very modest, may be helpful in recruiting and reassuring volunteers.

A disadvantage of the disposable forceps is cost. The disposable forceps cost between \$18.00 and \$60.00, depending on the manufacturer and on whether it is possible to negotiate a bulk discount. When procedures are done in endoscopy units with large volume, it may be easier to obtain competitive prices. There are costs associated with reusable forceps. The purchase

price is about six to seven times that of the disposable forceps (7). After each procedure, the forceps must be either steam autoclaved or manually cleaned and soaked in glutaraldehyde. Costs for the reusable forceps include technician/nurse salary and benefits, time needed to process the equipment, costs of the cleansing solution, and packaging. Factoring in the higher purchase price and the costs of processing, a reusable forceps had to be used at least seven times to be cost effective in a study by Kozarek *et al.* (7). If the disposable forceps can be obtained at a discounted rate, the number of uses for the reusable forceps would rise proportionately. In the Kozarek *et al.* (7) study, 42% of the forceps were used 10 or fewer times, and 36% were used between 11 and 20 times.

There are some limitations to this study. The comparisons were not made at the same point in time. Advances in forceps technology or mechanics over time could explain the results. However, the basic design of endoscopic forceps has not changed markedly during this interval. There are other features that distinguish forceps: the shape of the cups, sheaths, fenestrations, presence of a central needle, and teeth. During both parts of the study we used sheathed, oval, fenestrated forceps. The biopsies were taken by a number of different endoscopists with varying levels of training and experience. However, the biopsy technique is quite simple; in general, between-endoscopist variation is negligible. Furthermore, each part of the study took place over >1 years' time, which would serve to average effects of inexperience on the part of endoscopic trainees. Reusable forceps become dull over time. It is likely that there was drift in quality as forceps aged. This is reflected in the averages that we present. We do not have information on the performance of a specific forceps. The tissues were processed and read by different technicians. Although scoring of labeled cells might differ between readers, recognizing a crypt as scorable is less variable.

In summary, we found that the use of disposable forceps provided more adequate biopsies for a study of mucosal pro-

liferation. We believe that the disposable forceps were sharper and that the mechanism was more reliable. The disposable forceps also provide an added margin of safety against infection. Better biopsies, higher quality data, and reduced technician time balance the modest increase in the cost of the forceps. We believe that there has been inadequate attention in the literature to the forceps used for proliferation studies and would urge that investigators carefully evaluate the equipment used to obtain the tissue in biomarker studies.

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