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Histological assessment of new cholangioscopy-guided forceps in ERCP biliary stricture sampling: a blinded comparative study

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ABSTRACT

Background and study aims Obtaining quality tissue during ERCP biliary stricture sampling is of paramount importance for a timely diagnosis. While single-operator cholangioscopy (SOC)-guided biopsies have been suggested to be the superior biliary tissue acquisition modality given direct tissue visualization, less is known about the specimen histological quality. We aimed to analyze the specimen quality of SOC biopsies and compare the new generation forceps with prior "legacy" forceps.

Patients and methods Patients who underwent SOC from January 2017-August 2021 for biliary sampling were reviewed. In February 2020, the SOC-guided biopsy forceps were changed from legacy SpyBite to the SpyBite Max forceps (max). Specimens were assessed by blinded pathologists for crush artifact (none, mild, or severe) and gross size (greatest dimension in mm). Crush artifact and gross size were compared between the two groups. The diagnostic performance characteristics for cholangiocarcinoma (CCA), were assessed in an exploratory fashion.

Results Eighty-one patients (max = 27, legacy = 54) with similar baseline characteristics were included in this study. On blinded pathological assessment, 58 % had crush artifact, without significant differences between the two groups (Max 63 % vs. Legacy 56 %; $P = 0.64$). A similar mean specimen size was found (max 3 mm vs. legacy 3.2 mm; $P = 0.24$). The overall prevalence of CCA was 40 %. The sensitivity, specificity, positive predictive value, and negative predictive value of the entire cohort using a combination of cytology, fluorescence in situ hybridization, and SOC-guided biopsies were 78.1 %, 91.8 %, 86.2 %, and 86.5 %, respectively. No difference between legacy or max groups was found.

Conclusions A high rate of crush artifact was found in SOC-guided biopsy specimens. Further investigation regarding proper biopsy technique and handling is necessary to increase the diagnostic yield with SOC-guided biopsies.

Introduction

Obtaining a tissue diagnosis in indeterminate biliary strictures can be challenging, yet of paramount importance given the treatment implications when a malignancy is suspected. While the diagnosis of malignant strictures can often be predicted based on the clinical scenario and radiographic/cholangioscopic findings, tissue acquisition is required for a final diagnosis [1]. Endoscopic retrograde cholangiopancreatography (ERCP) is the procedural gold standard to obtain cells using brush cytology with associated molecular testing (fluorescence in situ hybridization [FISH]), and transpapillary fluoroscopy-guided biopsies but is hampered by varying levels of sensitivity and specificity throughout the literature [2–10]. Over the years, cholangioscopy has revolutionized ERCP with the ability to directly visualize the biliary tree with added diagnostic and therapeutic capabilities. As a result, single-operator cholangioscopy (SOC) has become increasingly popular for direct tissue visualization and tissue acquisition in indeterminate strictures. However, SOC-guided biopsies also carry their variability in performance characteristics throughout the literature [11–13]. In real-world practice, a combination of the above tests, occasionally over two or three procedures, leads to sufficient diagnostic certainty to pursue treatment [14, 15].

The reasons for variability in diagnostic performance characteristics are multiple, including endoscopist experience, equipment availability, tissue acquisition technique, and patient-level characteristics. While multiple studies have investigated the different equipment and techniques for brushings, less is known about the adequate technique for SOC-guided biopsies. Recently, it has been reported that obtaining at least 3 SOC-guided biopsies increases the odds of obtaining a timely diagnosis [11, 16]. However, even less is known about the biopsy specimen quality for histopathologic analysis. We aimed to analyze the biopsy specimen quality of SOC-guided biopsy specimens and compare the new SOC forceps design with the prior generation forceps in terms of biopsy specimen quality.

Patients and methods

Patient population

The study was approved by the Institutional Review Board at Mayo Clinic in Rochester, Minnesota (IRB 18–011272). Patients who underwent SOC for biliary sampling from January 2017-August 2021 were reviewed. In February 2020, the newly designed SOC forceps (SpyBite Max, Boston Scientific Corporation, Marlborough, Massachusetts, United States) became available and were used exclusively until the study's end. The new forceps incorporated several new design features compared to the prior generation forceps (SpyBite, Boston Scientific Corporation, Marlborough, Massachusetts, United States), namely teeth were added to the forceps as well as increased tissue capacity within the forceps cup, allowing for 2-fold more tissue in comparison (▶ **Fig. 1**) [17]. Additionally, a spike located in the center of the specimen cup was removed in the new iteration. The spike was initially intended to secure small tissue samples and allow for multiple bites without losing the specimen; however, it appeared to impede targeted tissue acquisition as the spike was noted to bounce off fibrotic tissue. Both forceps versions have an outer diameter of 1.0 mm, a jaw opening of 4.1 mm, and a working length of 286 cm.

▶ **Fig. 1** Newer generation SpyBite Max cholangioscopy forcep.

Pertinent clinical information such as demographics, primary sclerosing cholangitis (PSC) diagnosis, presence of a mass on cross-sectional imaging within 30 days of the procedure, and results from same session cytology, FISH, and transpapillary fluoroscopy-guided biopsy results were extracted from the electronic medical record. Brush cytology was obtained using a "to and fro" motion across the entire area of interest 20 times before removing the brush catheter from the patient. The specimen was sent for cytology and FISH in 15 mL of ThinPrep CytoLyt solution (Hologic, Marlborough, Massachusetts, United States). For transpapillary fluoroscopy-guided or SOC-guided biopsies, one to two bites were obtained per pass, with at least three biopsies performed per procedure. The transpapillary fluoroscopy-guided biopsies were obtained using single-use pediatric biopsy forceps (Radial Jaw 4 Pediatric Biopsy Forceps, Boston Scientific Corporation, Marlborough, Massachusetts, United States). Biopsy specimens were placed in standard formalin bottles for processing.

Outcome

The primary outcome of the study was the difference in the quality of SOC-guided biopsy specimens when comparing the new SOC forceps (max) and the prior generation forceps (legacy). The SOC-guided biopsy specimens were reviewed by blinded expert pathologists and graded on their crush artifact score (none, mild [< 5 %], or severe [> 30 %]), and gross size (greatest dimension in mm). Additionally, the performance characteristics of SOC-guided biopsies, cytology, molecular testing (FISH), and transpapillary fluoroscopy-guided biopsies for the detection of malignancy, specifically CCA, were assessed in an exploratory fashion. Cytology was considered positive if reported as suspicious for adenocarcinoma or overt malignancy was found. FISH was considered positive if polysomy (≥ 4 epithelial cells with gains of two or more loci: 1q21, 7p12, 8q24, and/or 9p21) was found. Biopsy specimens, whether through SOC forceps or transpapillary fluoroscopy-guided biopsy forceps were considered positive if suspicious of malignancy or overt malignancy was found.

▶ **Table 1** Baseline characteristics.

	Overall n = 81	Max n = 27	Legacy n = 54	*P* value
Age, years (mean ± SD)	60 ± 14.1	62.4 ± 16.1	58.4 ± 12.9	0.23
Sex, female	36 %	25 %	37 %	0.74
PSC dx	37 %	31.3 %	40.7 %	0.33
Mass on CT/MRI	25 %	37.5 %	22 %	0.47
CCA dx	40 %	50 %	61 %	0.87
Follow-up, months (median, IQR)	12.6 [8.4–23.2]	10.3 [5.1–13.1]	16.9 [8.9–31]	0.01

SD, standard deviation; PSC, primary sclerosing cholangitis; CT, computed tomography; MRI, magnetic resonance imaging; CCA; IQR, interquartile range.

Statistical analyses

Baseline demographics were compared between the two groups using the student's *t*-test for continuous variables and the Chi-Square test for categorical variables or their nonparametric equivalent. The crush artifact score and gross size were compared between the two groups using the Chi-Square test (or Fisher's exact, when appropriate) and student's *t*-test, respectively. The performance characteristics of SOC-guided biopsies, cytology, FISH, and transpapillary fluoroscopy-guided biopsies at detecting malignancy were reported as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). All continuous variables were expressed as mean and standard deviation (SD) and categorical variables as percentages. All tests were two-sided with $P < 0.05$. The analysis was performed using STATA 16 (StataCorp, College Station, Texas, United States).

Results

Baseline characteristics

A total of 81 patients (n = 54 in the legacy group, n = 27 in the max group) were eligible to be included in this study. Overall baseline characteristics were not significantly different between the two groups (▶ **Table 1**). The overall detection of CCA was 40 % (n = 32) in this cohort, without significant differences between the two groups (max 50 % vs. legacy 61 %; P = 0.87). The median length of follow-up after SOC-guided biopsies was 12.6 months [IQR 8.4–23.2] with a significant difference between the two groups (max 10.3 months [IQR 5.1–13.1] vs. legacy 16.9 months [IQR 8.9–31]; P = 0.01).

Quality assessment

On the blinded pathological assessment of the entire cohort, 58 % were noted to have crush artifacts, with 43 % being mild and 15 % severe. In the max group, 63 % had some crush artifact, with 41 % being mild and 22 % severe. In the legacy group, 56 % had some crush artifact, with 44 % being mild and 12 % severe. However, there were no significant differences between the two groups (▶ **Table 2**). In terms of specimen size, the gross size was 3.2 mm ± 1.5 mm without significant differences between the two groups (max 3 mm ± 1.2 mm vs. legacy 3.3 mm ± 1.6 mm; P = 0.24). When assessing the number of positive biopsies for malignancy, the proportion of positive biopsies was significantly higher in the max group at 25.9 % vs. 9.3 % (P = 0.047).

▶ **Table 2** Crush artifact and size.

	Overall n = 81	Max n = 27	Legacy n = 54	*P* value
Crush artifact				
None	42 %	37 %	44 %	0.64
Any	58 %	63 %	56 %	
Mild	43 %	41 %	44 %	0.41
Severe	15 %	22 %	12 %	0.41
Gross size, mm (mean ± SD)	3.2 ± 1.5	3 ± 1.2	3.3 ± 1.6	0.24

SD, standard deviation.

Performance characteristics

The sensitivity, specificity, PPV, and NPV of the entire cohort using a combination of cytology, FISH, and SOC-guided biopsies were 75 %, 91.8 %, 85.7 %, and 84.9 %, respectively (▶ **Table 3**). However, there were no significant differences in the area under the receiver operator curves between the max and legacy groups (area under the curve 0.82 vs. 0.62; P = 0.11).

Discussion

In this retrospective cohort assessment of SOC-guided biopsy specimens by blinded pathologists, there were several important findings. First, there was no significant difference in biopsy quality, whether assessing crush artifact or gross size between the new and older generation SOC biopsy forceps. Second, more importantly, there was a high crush artifact across all specimens in this cohort, highlighting the need to improve tissue acquisition technique and specimen quality in this population.

▶ **Table 3** Performance characteristics.

Test	Sensitivity	Specificity	PPV	NPV
Cytology	53.3 %	92.9 %	84.2 %	73.6 %
Cytology + FISH	56.7 %	97.6 %	94.4 %	75.9 %
Cytology + FISH + SOC biopsies	75 %	91.8 %	85.7 %	84.9 %

FISH, fluorescence in situ hybridization; SOC, single-operator cholangioscopy.

Third, when using a combination of cytology with FISH, and SOC biopsies diagnostic performance characteristics remained similar.

The importance of proper biopsy acquisition technique has previously been investigated in multiple upper endoscopy and colonoscopy studies looking at celiac disease and ulcerative colitis [18–20]. These studies have reported that a technique using a single biopsy per pass improved histological quality and reduced the likelihood of specimen loss. Conceivably, this technique is of even greater significance in biliary sampling, where the forceps used are smaller in size than traditional biopsy forceps. Currently, obtaining at least three SOC-guided biopsies is associated with improved diagnostic yield [16]. Given the high rates of crush artifact noted in this cohort, further investigation on whether "tissue stacking" or "one bite per pass" affect specimen quality.

To date, tissue sampling remains the gold standard for establishing a diagnosis of a malignant biliary stricture. While multiple studies have investigated patient and technical factors associated with improved odds of obtaining a diagnosis, such as strictures > 1 cm long, total bilirubin > 4 mg/dL, a mass on imaging, brushings pre-and post-dilation, brushing > 10 times, and obtaining at least three biopsies, there has been less focus on improving tissue quality [20–22]. Given the challenges of obtaining adequate tissue, other criteria have been established for a presumed clinical diagnosis of extrahepatic CCA in the absence of tissue diagnosis, including imaging features of a dominant stricture with an elevated serum CA19–9 > 129 U/mL, or other suspicious imaging findings (e. g. hilar mass or vascular encasement) [23]. SOC has been shown to improve the detection of malignant biliary strictures, yet the sensitivity of SOC-guided tissue sampling for the detection of malignancy remains lower than expected [24]. Much of the benefit of SOC has been the ability to detect visual features that are suggestive of malignancy. Whether other treating physicians would be accepting of an optical diagnosis remains to be determined.

Alternatively, novel biomarkers may be required to obtain a diagnosis, relying less on biopsy specimen quality, and more on obtaining tissue from the lesion of interest for genomic testing [25, 26]. Next-generation sequencing can be performed on standard brush cytology specimens; however, up to 11 % of brush samples may not yield enough DNA for testing [27]. Obtaining a second brush and/or biopsy sample dedicated to genomic testing may be needed, but whether the second sampling or genomic testing itself yields the higher sensitivity has not been prospectively investigated. Many molecular testing methods require adequate tumor tissue and cellularity with a minimum percentage of tumor nuclei per sample (e. g., 5 % to 10 %). While there is still limited data on the impact of crush artifacts on DNA or polymerase chain reaction testing, it negatively affects the ability to determine tissue adequacy [28]. The crushed tissue fragments also lose cellular and nuclear details required for FISH analysis [29]. For all these reasons, improving the quality of our biopsy specimens remains critical for both traditional and novel molecular testing methods.

There are several limitations to our study. First, given its retrospective nature, we were unable to assess biopsy techniques with granular detail, such as whether "one bite per pass" or multiple bites were taken in each patient, whether a prior dilation was performed or the order of tissue acquisition (brushed for cells first or after biopsies, for example) all which could alter the histologic yield based on prior studies [21, 22, 30–32]. In addition, the number of newer generation SOC forceps samples in this group was small compared to the older forceps, which was expected given the relatively recent introduction of these forceps to the marketplace and the need for adequate follow-up.

Conclusions

Overall, this study highlights that improving tissue quality, not just quantity is of paramount importance for the early detection of malignancy. By optimizing tissue quality, patients may receive an earlier diagnosis, which has the added benefit of reducing subsequent procedures and overall costs. Given the high rate of crush artifact observed with SOC-guided specimens, optimizing tissue quality even with traditional ERCP sampling techniques is warranted. Direct tissue visualization remains the greatest benefit of SOC and obtaining quality tissue is needed to bridge the current limitations of SOC. Thus, more studies on the optimization of tissue quality and specimen handling of SOC-directed biopsies for diagnostic testing are warranted.

Competing interests

Dr. Storm is a consultant for Apollo Endosurgery and received research support from Apollo Endosurgery and Boston Scientific.
Dr. Law is a consultant for ConMed and Medtronic and receives royalties from UpToDate.
Dr. Abu Dayyeh reports consultant roles with Endogenex, Endo-TAGSS, Metamodix, and BFKW; consultant and grant or research

support from USGI, Cairn Diagnostics, Aspire Bariatrics, Boston Scientific; speaker roles with Olympus, Johnson and Johnson; speaker and grant or research support from Medtronic, Endogastric solutions; and research support from Apollo Endosurgery and Spatz Medical.
Dr. Petersen is a consultant for Olympus America and investigator for Boston Scientific and Ambu.
Dr. Chandrasekhara is a consultant for Covidien LP and Boston Scientific and is a shareholder in Nevakar Corp.
The remaining authors have no conflicts or funding to disclose.

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