Cholangioscopy and Cholangioscopic Forceps Biopsy in Patients With Indeterminate Pancreaticobiliary Pathology

RAJ J. SHAH, DANIEL A. LANGER, MAINOR R. ANTILLON, and YANG K. CHEN

Division of Gastroenterology and Hepatology, Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado

Background & Aims: We report the usefulness of cholangioscopy in patients with indeterminate pancreaticobiliary pathology.

Methods: A prospective collection of 62 consecutive patients during a period of 2.5 years who were referred to our tertiary referral center for cholangioscopy for indeterminate strictures suspicious for malignancy were included. Tissue sampling followed cholangioscopic visualization. Biopsies were obtained under direct visualization (cholangioscopy-directed) or through the duodenoscope (cholangioscopy-assisted).

Results: Sixty-two patients had 72 examinations. Forty patients had nondiagnostic sampling before cholangioscopy. Indications were stricture (n = 67: 16 primary sclerosing cholangitis, 51 non–primary sclerosing cholangitis), ductal dilation, or intraductal mass (n = 5). Biopsies were not performed in 19 because cholangioscopy did not identify suspicious lesions. Of the remaining 53 procedures, 29 underwent either cholangioscopy-directed or cholangioscopy-assisted biopsy, and 24 had both. Cholangioscopy findings consisted of primary sclerosing cholangitis only (n = 18), benign stricture or inflammatory changes (n = 18), bile duct cancer (n = 14), normal (n = 10), pancreatic cancer (n = 5), and other (n = 7). Fiftysixeight patients (94%) had follow-up for a mean of 12.4 months (95% confidence interval, 10.1–14.7). Sixteen of 18 (89%) patients with a final diagnosis of malignancy were detected with cholangioscopy. The 2 missed cancers were intrahepatic cholangiocarcinomas. Overall, sensitivity to detect malignancy by cholangioscopy with and without biopsy was 89%, specificity 96%, positive predictive value 89%, and negative predictive value 96%.

Conclusions: Cholangioscopy with and without biopsy is highly accurate in diagnosing and excluding pancreaticobiliary malignancy in patients with indeterminate strictures.

Tissue sampling at endoscopic retrograde cholangiopancreatography (ERCP) is dependent on accurate localization of pathology on the basis of the fluoroscopic appearance of a stricture or an intraductal lesion. If pre-sampling dilation of strictures is performed, contrast might empty from the ductal system, thus further limiting the area to be sampled. In patients with diffuse involvement, such as primary sclerosing cholangitis (PSC), it might be more difficult to decide where to biopsy. The sensitivity of tissue sampling obtained during ERCP depends on several factors. Whether “atypical” or “suspicious” results are assumed to be malignant, the location and type of malignancy, and whether triple-tissue sampling methods (eg, brush cytology, biopsy forceps, and fine-needle aspiration [FNA]) are used are important considerations when assessing its yield. Given these variables, ERCP’s sensitivity for detecting malignancy varies from 30%–70% in selected series.

Recently, in patients with indeterminate biliary strictures, high-frequency intraductal ultrasound has been used as an adjunct to tissue sampling for assessing vessel invasion, length of the stricture, or asymmetry. However, its use is limited by the inability to obtain tissue samples. Cholangiopancreatoscopy (CP) has the advantages of direct visualization of the stricture or filling defect noted on fluoroscopy and inspection of the biliary epithelium for subtle abnormalities that might not be detected radiographically. With the availability of miniature cholangioscopic biopsy forceps, directed tissue sampling is feasible.

The percutaneous transhepatic approach was initially required for cholangioscopic evaluation of biliary strictures. Recent refinements in optics, scope diameter, and flexibility have enabled endoscopists to advance the cholangioscope through the operating channel of a standard therapeutic duodenoscope, thus permitting peroral transpapillary intubation without a dedicated “mother-daughter” system. CP might alter patient management by confirming or excluding sus-
pected malignancies; treating difficult pancreatic, extrahepatic, and intrahepatic stones; providing selective access to a diseased segment; and planning additional intervention.\textsuperscript{10} There is a paucity of published literature on the yield of per oral cholangioscopic-directed biopsies. To our knowledge, this is the largest consecutive series of directed tissue sampling with per oral cholangioscopy and cholangioscopy-directed forceps biopsy (CDB) in patients with indeterminate pancreatic or biliary pathology.

**Patients and Methods**

Consecutive patients referred to the University of Colorado Hospital between May 2000 and November 2002 for suspected pancreatic or biliary malignancy primarily on the basis of previous ERCP findings were included. Institutional Review Board approval was obtained for the collection of patient data and follow-up. During the study period, CP was performed whenever ERCP suggested a stricture, intraductal lesion, or had equivocal fluoroscopic findings. A data collection instrument was used to record patient demographics, indications, prior interventions and noninvasive imaging studies, prior attempts at tissue sampling, endoscopic and histopathologic findings, subsequent interventions, final diagnosis, and endoscopic complications defined by published consensus criteria.\textsuperscript{11} CP diagnosis was based on visual inspection with or without histologic confirmation. Follow-up was attempted in all patients. Final diagnosis was based on endoscopic or surgical pathology and/or clinical course.

Cholangioscopy was performed via a peroral approach by using a standard therapeutic duodenoscope with a 4.2-mm operating channel (TJF140 or TJF160; Olympus, Melville, NY) or occasionally through an established percutaneous tract (Figure 1). The following cholangioscopes were used (Table 1): Olympus BP30 (fiberoptic), Pentax FCP9P (fiberoptic; Pentax, Orangeburg, NY), and Olympus CHF B160 (video). The scope diameter ranged from 3.1–3.4 mm with an operating channel of 1.2 mm. The Olympus video scope had a slightly smaller tip deflection (90 degrees up and down) when compared with the fiberoptic instrument (160 degrees up and 130 degrees down).

Two endoscopists performed all the procedures during the study period (R.S. and Y.C.). Most of the patients received antibiotic prophylaxis, usually levofloxacin 500 mg intravenous. After cholangiopancreatography and placement of a 0.035-inch guidewire, the cholangioscope was advanced over the guidewire into the biliary and/or pancreatic ducts. Sphincterotomy and stricture dilation were performed as needed to facilitate scope passage across a lesion. If a percutaneous transhepatic drain was previously placed, then cholangioscopy was performed through an established percutaneous tract with a 12F sheath. To enhance visualization and permit use of the operating channel of the cholangioscope, the guidewire was removed, and sterile saline was intermittently flushed through the operating channel of the cholangioscope, followed by a slow withdrawal of the scope during systematic inspection of the biliary or pancreatic system.

**Procurement of Specimens**

CDB was performed whenever CP inspection revealed any of the following: intraductal nodules or masses, infiltrative or ulcerated strictures, and papillary or villous mucosal projections (Figure 2).\textsuperscript{12} Biopsies were not performed if erythematous epithelial changes were not raised or associated with a defined stricture. If fluoroscopic findings were equivocal (eg, ductal dilation with suspected distal biliary stricture) but the CP was normal, then biopsies were not obtained.

CDB was performed by using a miniature cholangioscope biopsy forceps (Olympus) under direct endoscopic visualization. For cholangioscopy-assisted biopsy (CAB), the target biopsy site was localized by using CP visualization and a fluoroscopic spot film of the cholangioscope tip position at the lesion. This was followed by the passage of a conventional biopsy forceps through the operating channel of the duodenoscope to obtain tissue samples under fluoroscopic guidance. The decision to perform both methods of tissue sampling was at the discretion of the endoscopist. Examples of reasons as to why both methods might not have been used on a given patient are if the miniature biopsy forceps could not be passed through the working channel of the cholangioscope because of acute angulation of the cholangioscope, or if tissue samples acquired by using CDB were deemed to be adequate by visual inspection, then CAB might not have been pursued. A minimum of 3 passes and bites were attempted with each biopsy method.\textsuperscript{15}

**Statistics**

Surgical confirmation of the etiology of the stricture could not be obtained in all patients; therefore, a composite reference standard that included the results of tissue sampling and clinical course at time of follow-up was used for confirmation of the final diagnosis. Sensitivity, specificity, negative predictive values, and positive predictive values were calculated on the basis of this reference standard, as previously described.\textsuperscript{14} Standard deviation and 95% confidence intervals were calculated by using standard formulas.

**Results**

Sixty-two consecutive patients (33 men and 29 women) with a mean age of 61.2 years (range, 27–87 years) underwent 76 CP examinations during the study period. Four of these patients had repeat procedures to treat biliary stones. Thus, 72 examinations (8 percutaneous) were performed to assess for indeterminate or suspected strictures and constitute the basis of this report. Before the index CP, 56 of 62 (90%) patients had
a mean of 2.1 ERCPs (range, 1–9), and 49 (79%) had nondiagnostic imaging studies by computed tomography, magnetic resonance imaging, or positron emission tomography scanning. Forty of 62 (65%) patients had previous nondiagnostic tissue sampling: 30 had ERCP brush cytology, 8 had ERCP brush cytology and biopsy, 1 had ERCP with triple-tissue sampling, and 1 patient had endoscopic ultrasonography (EUS)–FNA alone.

Eight of these 40 patients had both EUS-FNA and ERCP tissue sampling before index CP.

Indications for CP included the evaluation of both PSC and non-PSC strictures and are shown in Table 2. Nineteen CP examinations did not identify a suspicious lesion or stricture; therefore biopsies were not performed. Of the remaining 53 examinations, 24 (45%) underwent both CDB and CAB. In total, there were 42 CDBs and 35 CABs obtained. One patient had cholangioscopy-directed brush cytology and cholangioscopy-assisted brush cytology. CP diagnoses were based on CP visualization with or without histologic confirmation (Table 3). Diagnoses included PSC without cholangiocarcinoma, benign strictures, and bile duct cancers (Table 3). Follow-up was achieved in 58 patients (94%) during a

Table 1. Cholangioscope Usage

<table>
<thead>
<tr>
<th>Scope type</th>
<th>Number of cases (N = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olympus CHF BP 30 (fiberoptic)</td>
<td>54</td>
</tr>
<tr>
<td>Olympus B160 (video)</td>
<td>10</td>
</tr>
<tr>
<td>Pentax FCP9P (fiberoptic)</td>
<td>8</td>
</tr>
</tbody>
</table>

Figure 1. (A) Cholangiogram of a patient who had 3 previous attempts for a tissue diagnosis at an outside hospital. Fluoroscopy reveals a common hepatic duct and hilar stricture. (B) Fluoroscopy view of the cholangioscope at the common hepatic duct with open biopsy forceps through the working channel. (C) Cholangioscopic view of the biopsy forceps. (D) Cholangioscopic view of a malignant-appearing circumferential stricture. Biopsies revealed poorly differentiated adenocarcinoma.
mean of 12.4 months (95% confidence interval, 10.1–14.7). Of the 4 patients lost to follow-up, 2 had tissue confirmation of cholangiocarcinoma by cholangioscopy, 1 patient had suspected PSC based on 4 prior percutaneous transhepatic cholangiographies (intrahepatic strictures) and negative biopsies at time of cholangioscopy, and 1 patient had an ERCP finding of common hepatic duct stricture before cholecystectomy but a normal cholangioscopy postoperatively, and the likely etiology of the stricture that prompted referral was Mirizzi’s syndrome.

The final diagnoses are described in Table 4. Complications occurred during 4 CP examinations (5.6%): pancreatitis (1 mild, 1 moderate), cholangitis (mild), and perforation related to sphincterotomy (severe).

Adequacy of Histologic Specimens

Thirty-nine of 42 (93%) of the CDBs and all of the CABs were deemed to be adequate specimens for diagnosis. The 3 patients who had inadequate CDB are described. CP findings in 1 patient were believed to be most consistent with PSC without malignancy, and the patient has no evidence of cancer at 13 months of follow-up. A second patient with a malignant-appearing common hepatic duct stricture by CP and negative CAB subsequently underwent EUS-guided FNA cytologic confirmation of cholangiocarcinoma at the hilum. The third patient had a repeat CP to biopsy a previously visualized intraductal mass; histology showed a large B-cell lymphoma of the common hepatic duct.

Table 3. CP Diagnoses

<table>
<thead>
<tr>
<th>Findings</th>
<th>Number of patients</th>
<th>Number of examinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSC</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Benign stricture/inflammatory changes</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Pancreatic adenocarcinoma</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Benign intraductal polyp</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Bile duct lymphoma</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Squamous cell cancer</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Intraductal papillary mucinous tumor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Obstructing biliary calculi</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Papillary stenosis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>62</td>
<td>72</td>
</tr>
</tbody>
</table>

Two patients presumed to have cholangiocarcinoma on index CP were subsequently diagnosed on follow-up CP as radiation-induced strictures and PSC, respectively.

Histologic confirmation at 2nd CP.

Table 4. Final Diagnoses

<table>
<thead>
<tr>
<th>Final diagnosis</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSC</td>
<td>18</td>
</tr>
<tr>
<td>Benign biliary stricture</td>
<td>15</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>11</td>
</tr>
<tr>
<td>Normal</td>
<td>9</td>
</tr>
<tr>
<td>Pancreatic adenocarcinoma</td>
<td>4</td>
</tr>
<tr>
<td>Bile duct lymphoma</td>
<td>1</td>
</tr>
<tr>
<td>Intraductal papillary mucinous tumor</td>
<td>1</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Benign intraductal polyp</td>
<td>1</td>
</tr>
<tr>
<td>Papillary stenosis</td>
<td>1</td>
</tr>
<tr>
<td>Total number of patients</td>
<td>62</td>
</tr>
</tbody>
</table>
In the 24 examinations in which both CDB and CAB were obtained, histologic diagnosis was identical in 22 (92%) tissue samples with both methods. One patient with hilar and intrahepatic strictures had adequate tissue samples by both methods, but only the CAB was positive for cholangiocarcinoma because of the inability to advance the cholangioscope beyond the hilar stricture to the area of interest. The other patient had CP visualization of a malignancy but an inadequate CDB and a false-negative CAB; EUS-FNA was required to establish the final tissue diagnosis of malignancy.

Brush cytology was not systematically performed in this study; however, 9 examinations included duodenoscopic brush cytology with fluoroscopic guidance either before or after CP visualization (1 inadequate sample, 3 negative, 5 atypical cells). On the basis of follow-up and CP examination, if atypical cytology is taken to be negative for malignancy, then of the 9 ERCP brush cytology specimens, 1 was inadequate, 5 were true-negatives, and 3 were false-negatives.

**Comparison of the Cholangiopancreatoscopy Diagnosis With the Final Diagnosis**

Discrepancy between the CP diagnosis and the final diagnosis was based on subsequent diagnostic imaging or interventions such as computed tomography, magnetic resonance imaging, positron emission tomography, surgery or EUS, and clinical course during a mean follow-up of 12.4 months. Of the 18 patients with a final diagnosis of malignancy (Table 4), 11 (61%) had prior nondiagnostic tissue sampling by ERCP. CP diagnosed 16 of the 18 malignancies (89%), but 2 intrahepatic cholangiocarcinomas were missed. One patient with atypical cells on brush cytology by ERCP had a normal CP examination limited by an intrahepatic stricture; cholangiocarcinoma was found intraoperatively in the caudate lobe. The second missed cancer was a PSC patient with negative CDB and CAB; cholangiocarcinoma was found at the hilum of the explanted liver 7 months later. The latter patient is still alive at 35 months of follow-up. Two other patients were thought to have hilar cholangiocarcinoma by cholangioscopic visualization, but both CDB and CAB were negative for malignancy; subsequent intraoperative biopsies were also negative, and both are without clinical evidence of malignancy at 12 and 19 months of follow-up, respectively. All 4 patients with discordant results between the CP diagnosis and the final diagnosis had intrahepatic strictures.

Of the 16 patients with a CP diagnosis of malignancy, CDB and/or CAB were confirmatory in 10 patients (63%). Histologically, 6 had cholangiocarcinoma, and 1 patient each had squamous cell carcinoma, primary bile duct lymphoma, pancreatic adenocarcinoma, and intraductal papillary mucinous tumor. The pathology findings were positive in 9 of 10 CDB biopsies and in all 10 CAB biopsies. In 5 of these 10 patients with a malignancy diagnosed by CP biopsies, prior attempts at tissue sampling by ERCP had failed to establish the diagnosis. Of the remaining 6 patients with a CP diagnosis of malignancy but nondiagnostic biopsies, the final diagnosis of malignancy was established by tissue sampling with other methods and clinical course. Overall, the sensitivity to detect malignancy by cholangioscopy with and without biopsy in this cohort of patients with indeterminate pancreaticobiliary pathology was 89%, specificity 96%, positive predictive value 89%, and negative predictive value 96%.

**Discussion**

Since its inception, the role for ERCP continues to expand and includes diagnosing and treating both benign and malignant pancreaticobiliary strictures and calculi. However, cholangiopancreatography is often equivocal for diagnosis of stones and strictures, and conventional tissue sampling during ERCP might be nondiagnostic despite a high clinical suspicion. To avoid treatment delay and offer the appropriate definitive medical or surgical therapeutic intervention, it is important to aggressively pursue tissue confirmation of suspected malignancies. CP can now assist in the investigation of indeterminate lesions.

Initially, the percutaneous approach was favored because cholangioscopes were larger than the operating channel of a conventional therapeutic duodenoscope. Subsequently, cumbersome mother-daughter systems were developed to permit the introduction of the cholangioscope via the transpapillary approach. The per oral approach with and without the use of a mother scope was first described by Kawai et al in 1976, followed by several reports from both Japan and Europe. Because of the requirement for a mother-daughter system, dedicated processor, and fragility of the miniature scope, availability was limited, and the technology was not widely adopted. Later-generation endoscopes with a smaller diameter and up-down angulation permitted the advancement of the daughter scope through the working channel of a conventional therapeutic duodenoscope.

Both peroral and percutaneous cholangioscopy have become established as an additional modality for the therapy of difficult intrahepatic and extrahepatic calculi by using an electrohydraulic fiber or laser to perform lithotripsy. However, there are very few reports on
the utility of cholangioscopy for evaluating biliary strictures. There is a single report of peroral cholangioscopy with biopsy; however, a cumbersome mother-daughter system was required, and the series only included selected cases. The yield of CDBs via the percutaneous system was required, and the series only included with biopsy; however, a cumbersome mother-daughter

In our series, CDB provided positive histology in 10 of the 16 patients in whom CP visualization was consistent with malignancy. These included cholangiocarcinoma (N = 6), primary squamous cell carcinoma (N = 1), bile duct lymphoma (N = 1), pancreatic adenocarcinoma (N = 1), and intraductal papillary mucinous tumor (N = 1). Two patients with cholangiocarcinoma located in the intrahepatic ducts were missed by cholangioscopy.

The largest published series on the role of peroral cholangioscopy in managing biliary tract diseases was reported by Siddique et al. However, one third of the 60 examinations were performed in liver transplant patients, mostly to evaluate anastomotic strictures. Only 11 patients had cholangioscopy to evaluate equivocal fluoroscopic findings or suspected cholangiocarcinoma. Cholangiocarcinoma was confirmed in 2 patients by brush cytology passed through the cholangioscope. However, the total number of patients who underwent cholangioscopic brush cytology was not described. CDB was not performed. Furthermore, prior attempts at tissue sampling and location of the cancer within the biliary tree were not described.

Our series included consecutive patients with indeterminate pancreaticobiliary pathology referred for CP primarily to exclude or confirm malignancy. Two thirds of the 62 patients evaluated by CP had prior nondiagnostic tissue sampling by ERCP. A diagnosis of malignancy was established on the basis of CP in 16 patients. Histologic confirmation of malignancy with CP-directed and/or assisted biopsies was obtained in 10 of the 16 patients, 50% of whom had prior nondiagnostic sampling. Two rare bile duct malignancies, squamous cell cancer and primary lymphoma of the bile duct, were diagnosed by CP-directed biopsies that altered the medical management and prognosis. On the other hand, 2 cancers were missed, and these were both intrahepatic cholangiocarcinomas that were beyond the reach of the 10F cholangioscope. Overall, sensitivity to detect malignancy by cholangioscopy with and without biopsy was 89%, specificity 96%, positive predictive value 89%, and negative predictive value 96%.

One limitation of this study is that the majority of patients referred for CP had brush cytology alone before CP (9 had biopsies along with brush cytology). It is possible that triple-tissue sampling during ERCP might have established the diagnosis in some of these patients, thus obviating the need for cholangioscopy. However, given the additional time and technical skill required for triple-tissue sampling, this is often not done in most busy endoscopy practices. Our referral population likely reflects that of most tertiary referral centers. Prospective studies would be required to determine whether cholangioscopy and CDB have a superior yield to repeat ERCP and triple-tissue sampling (or EUS with FNA) for confirming and excluding pancreaticobiliary malignancy in patients with initial indeterminate pathology.

Tissue sampling to exclude cholangiocarcinoma remains a challenge in patients with PSC. Sampling accuracy is complicated by the multicentric nature of this disease and an infiltrative type of cancer that might elude diagnosis by epithelial biopsy. Tumor markers such as CA 19-9 and carcinoembryonic antigen have been proposed to aid in screening patients for cholangiocarcinoma. In our series, cholangioscopy excluded cancer in 17 PSC patients at a mean follow-up of 17.5 months (95% confidence interval, 12.6–22.4), and 1 was lost to follow-up. Two patients with hilar strictures did not have a diagnosis of PSC before cholangioscopy, and their final diagnosis was based on each patient having 2 negative EUS examinations and 2 cholangioscopies during 24 months of follow-up; 1 of the 2 has since undergone liver transplant without cancer in the explanted liver. One of the 17 patients had an incidental cancer detected in the explanted liver that was missed by cholangioscopy, but the patient is alive at almost 3 years of follow-up. There are no published studies comparing the usefulness of cholangioscopy versus conventional ERCP as a screening strategy in PSC patients awaiting liver transplantation.

Pancreatocopy with or without biopsy might be used to distinguish chronic pancreatitis from pancreatic cancer. Most centers have preferred to use EUS for this clinical dilemma. For mucin-producing tumors of the pancreas, direct-visualization has aided in localization and determination of more dysplastic lesions in the main pancreatic duct to guide surgical therapy. Our single patient with intraductal papillary mucinous tumor proceeded to successful surgical resection (Figure 3).

Scope fragility, costs of repair, procedure time, personnel, and a lack of a specific reimbursement code for per oral cholangioscopy currently discourage the widespread use of CP. Significant technical difficulties remain with passing a biopsy forceps or lithotripsy fiber through the operating channel of the scope, and this relates to sharp angulation at the distal end of the duodenoscope.
when positioned at the papilla. Aggressive manipulation of the forceps within the operating channel also might contribute to scope damage and expensive repairs.

In summary, we have presented the largest consecutive series of CP and biopsy for the evaluation of patients with indeterminate biliary and pancreatic strictures. The results indicate that per oral CP and cholangioscopic biopsies are feasible, allow adequate and directed tissue sampling, and have an excellent predictive value for confirming and excluding malignancy in patients with indeterminate strictures or equivocal ERCP findings. Future developments in scope technology such as 4-way deflection, smaller diameter, a separate irrigation channel, and better ancillary devices will likely continue to improve intraductal access, localization of lesions, diagnostic accuracy, and application of novel therapeutic modalities.

References


Address requests for reprints to: Yang K. Chen, MD, University of Colorado Hospital, AOP, Gastroenterology, PO Box 6510, mail stop F735, Aurora, CO 80045. e-mail: Yang.Chen@UCHSC.edu; fax: 720-848-2749.

Dr Chen has received research grants and educational grants from Olympus.