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Title:

DIAGNOSTIC YIELD OF CHOLANGIOSCOPY, CONFOCAL LASER ENDOMICROSCOPY, INTRADUCTAL ULTRASOUND, BIOPSIES AND STANDARD CYTOLOGY IN INDETERMINATE BILIARY STRICTURES: A SIDE-BY-SIDE, PROSPECTIVE EVALUATION.

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ABSTRACT

INTRODUCTION AND AIMS: Evaluation of biliary strictures remains challenging, due to the low sensitivity of standard diagnostic work up. The dilemma that exists is how to balance the risk of failing to detect malignancy and the potential morbidity caused by unnecessary surgery in patients with benign etiologies.
Aim of our study was to evaluate diagnostic performance of digital single operator cholangioscopy (DSOC) and DSOC-targeted biopsies, probe-based confocal laser endomicroscopy (pCLE), intraductal ultrasound (IDUS) and brush cytology in patient with indeterminate biliary stricture (IBS); secondary aims were to evaluate the impact of biliary stenting in diagnostic accuracy of diagnostic techniques and the safety profile of DSOC.

PATIENTS AND METHODS: This was a prospective, monocentric, observational study involving patient underwent advanced diagnostic work up for IBS in our endoscopy unit, from '01/2018 to '09/2022; all patients had at least one previous attempt to characterize the biliary stricture with ERCP and/or EUS. All procedures were performed by high experienced endoscopists, in hospitalized patients underwent antibiotic prophylaxis. Operating characteristics were calculated and compared between techniques. Final diagnosis was based on surgical pathology (where available) and/or clinical and radiological follow-up of at least 6 months.

RESULTS: A total of 57 patients with a mean age of 67.2 ± 10.0 years were enrolled. The mean follow up was 18.2 ± 18.1 months. IBS were mostly located in the distal common bile duct (45.6%) and final diagnosis was consistent with malignancy in 35 patients (61.4%), with cholangiocarcinoma as more frequent etiology (27 patients).
DSOC, pCLE and IDUS showed a significantly higher accuracy (89.5%, 85.2% and 82.7% respectively) compared to standard cytology (61.5, $p < 0.05$). Thirty patients (52.6%) had a biliary stent in place, which did not significantly reduced the diagnostic accuracy of DSOC, pCLE and IDUS. Adverse events (AEs) rate was 17.5%, with cholangitis as more common complication (8.8%); all but one AEs were mild to moderate and managed with medical therapy; one case of cholangitis required a new endoscopic treatment with ERCP and stent replacement.

CONCLUSIONS : In this monocentric observational study, DSOC visualization, pCLE and IDUS demonstrated an optimal diagnostic yield in the differentiation of IBS, with an acceptable safety profile both quantitatively as qualitatively.

INTRODUCTION

Indeterminate biliary strictures (IBS) are regarded as such when the standard diagnostic work up turn out to be inconclusive, representing a diagnostic challenge for physicians. Standard diagnostic work-up includes cross sectional imaging with computer tomography (CT)-scan and magnetic resonance imaging (MRI), endoscopic retrograde cholangiopancreatography (ERCP) with brush cytology and/or endoscopic ultrasound (EUS) with fine needle aspiration (FNA) or biopsy (FNB).
Despite the application of the above-mentioned techniques, even in combination, biliary strictures can be easily mischaracterized and remain indeterminate in up to 20% of cases; moreover, one out of four surgically resected IBS demonstrate a benign histology.[1,2] In this setting, physicians and patients must carefully weigh the malignant potential of an IBS (which requires a fast diagnosis and prompt treatment) against the possibility of a benign etiology, for the high risk of mortality and morbidity of unnecessary surgery.[3]
The limits of standard diagnostic work up can lead to multiple, repeated procedures in order to obtain a diagnosis, which means extended time from clinical presentation to treatment (reducing the probability of a curative resection in patients with malignancy), but also increased risk for procedure-related adverse events; ERCP, the most common diagnostic procedure in IBS, can be complicated by acute pancreatitis (5.3 – 9.7%), cholangitis (0.8%) and perforation (0.09-1.67%). [4–7]

ETIOLOGY

Differential diagnosis involves both benign and malignant conditions, as reported in table 1. Since the majority of IBS do turn out to be malignant,[8] is essential to rule out cancer, specially pancreatic cancer and cholangiocarcinoma which are the most prevalent lesions in distal and proximal bile ducts, respectively.[1] Other malignancies that can present as IBS are ampullary adenocarcinoma, gallbladder cancer infiltrating the bile ducts, lymphoma, hepatocellular carcinoma and metastasis.
Iatrogenic injury during hepatobiliary surgery is the most common cause of benign biliary stricture, especially after cholecystectomy and orthotopic liver transplantation; furthermore, several inflammatory conditions (primary sclerosing cholangitis, IgG4 cholangitis, protracted bile duct lithiasis) can affect the biliary tree presenting as IBS.

Table 1. Etiology of biliary strictures.

Benign conditions	Malignant conditions
Iatrogenic and non-iatrogenic bile duct injury Post-cholecystectomy injury Anastomotic and non-anastomotic stricture after liver transplantation Partial hepatectomy Hepaticojejunostomy Chemotherapy Post-radiation therapy Trauma **Inflammatory conditions** Primary Sclerosing cholangitis IgG4 related cholangitis Eosinophilic cholangitis Mast cell cholangitis Histiocytosis X Sarcoidosis **Intraluminal obstruction** Bile duct stones **Extraluminal compression** Cholelithiasis (Mirizzi syndrome) Chronic pancreatitis Portal cholangiopathy Sphincter of Oddi disfunction **Vascular diseases** Ischemic cholangiopathy Vasculitis **Infectious diseases** Tuberculosis, histoplasmosis, parasitic, virus HIV cholangiopathy Recurrent pyogenic cholangitis	Pancreatic cancer Cholangiocarcinoma Ampullary adenocarcinoma Hepatocellular carcinoma Lymphoma Gallbladder cancer infiltration Metastasis

Clinical presentation

Clinical presentation of IBS can be broad, with a spectrum of manifestations ranging from incidental radiologic finding in asymptomatic patient to acute cholangitis with septic shock. The most common presentation is painless jaundice, that associated with malaise, fatigue and weight loss can suggest an underlying malignancy.
Clinical history can provide insights to specific etiology; for instance, a previous cholecystectomy increase the odds of a post-surgical stricture, and anastomotic biliary strictures can arise after liver transplants. Patient with primary sclerosing cholangitis have often a concomitant diagnosis of inflammatory bowel disease, and can present signs and symptoms of chronic liver diseases and portal hypertension; finally, patient with IgG4-related cholangitis can have a history of autoimmune pancreatitis. [9] The use of serum markers is limited by a poor specificity: IgG4 may be elevated also in cholangiocarcinoma and primary sclerosing cholangitis, and an increased carbohydrate antigen 19-9 can be seen in patient with pancreatic cancer and cholangiocarcinoma, but also in cholangitis, biliary obstruction and liver diseases. [10]

The role of radiological imaging

Transabdominal ultrasound represents the first diagnostic step when a biliary disorder is suspected,[11] because of its reasonable cost and low invasiveness, coupled with the extended availability. This technology has very high sensitivity in detecting intra- and extrahepatic duct dilation, but the direct visualization of the lesion can be difficult. The limited capacity to detect strictures or masses makes ultrasonography a good screening test that has to be followed by more sensitive imaging techniques, such CT scan and MR cholangiopancreatography (MRCP).[1]
CT scan has a greater sensitivity for detecting biliary strictures and associated masses, compared to transabdominal ultrasound; moreover, provides useful information about local and vascular invasion, nodal involvement and it can identify distant metastasis.[12]
MCRP has the capability to provide cholangiograms, which aid in the evaluation of location and extent of biliary stricture; it is noninvasive and it does not requires injection of contrast medium in the biliary tree, reducing the risk of bacterial translocation and subsequent cholangitis development. One of the main disadvantage of MRCP is the inability to obtain tissue sample for cytological and histological evaluation.[13]
Both CT scan and MRCP have high sensitivity but low specificity in the identification of IBS, which makes mandatory the endoscopic approach in order to obtain a diagnosis.[14]

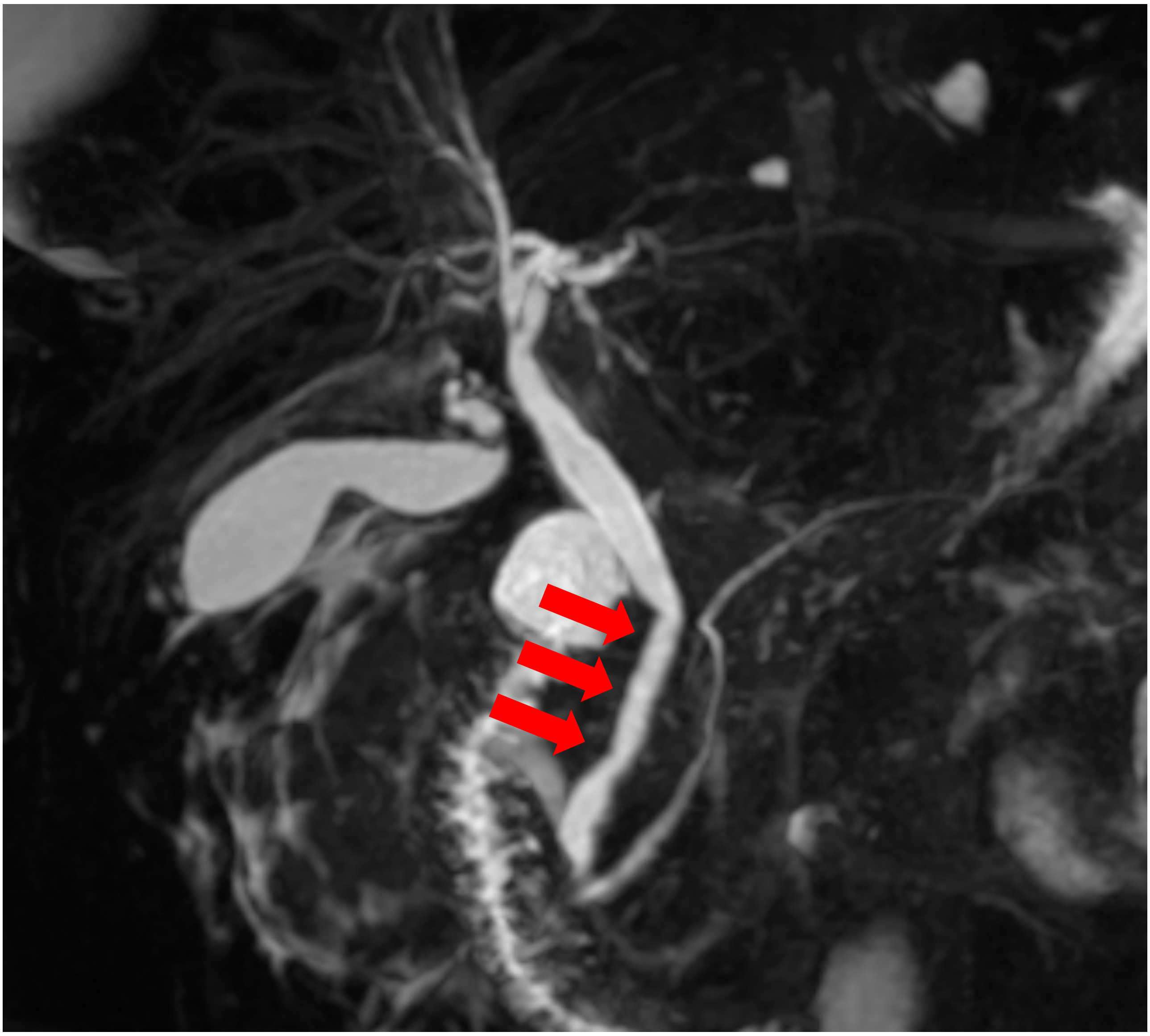

Figure 1. Magnetic resonance cholangiopancreatography of a biliary stricture in the common bile duct (red arrows)

THE ROLE OF ENDOSCOPY

Endoscopy has a prominent role in the characterization of IBS, permitting tissue acquisition and also therapeutic maneuvers such as biliary stenting.

Cholangiograms obtained during ERCP are useful to determinate morphology and extent of biliary strictures, and a tissue diagnosis can be obtained during the same procedure.

The two most common methods of tissue acquisition (brushing cytology and fluoroscopy-guided forceps biopsies) have unsatisfactory sensitivity of 25-50%; [12,15,16] also the combination of the two techniques increases the sensitivity to no more than 60%, as demonstrated by Navaneethan and colleagues.[17]

Figure 2. Endoscopic retrograde cholangiopancreatography of a biliary stricture.

The diagnostic role of EUS is significantly affected by the location of the stricture: in distal strictures, where pancreatic cancer is the most prevalent etiology, EUS with FNA/FNB has a sensitivity of 86% and a specificity of more than 95%;[18] on the other hand, intrahepatic ducts and hilum are less readily assessed by this technique.[19]

When ERCP is inconclusive, EUS plus FNA/FNB has an added diagnostic value, allowing the visualization of extrahepatic biliary tree, vessels and perihilar lymph nodes; it can also assist in tissue sampling when it may be technically difficult to do so with ERCP.

In two studies, EUS-FNA following negative or unsuccessful ERCP-guided tissue sampling was found to have a sensitivity of 77-89% , suggesting a reliable role for EUS in conjunction with ERCP;[19,20] moreover, a meta-analysis by Chiang and colleagues on more than 1100 patients has demonstrated an incremental benefit of EUS (described as the proportion of patient where ERCP did not yield a diagnosis of malignancy, but EUS did) of about 14%.[21]

A major limitation in the use of EUS-guided FNA/FNB is a not negligible risk of tumor seeding when a transperitoneal route is necessary to reach hilar or intrahepatic lesions.[22]

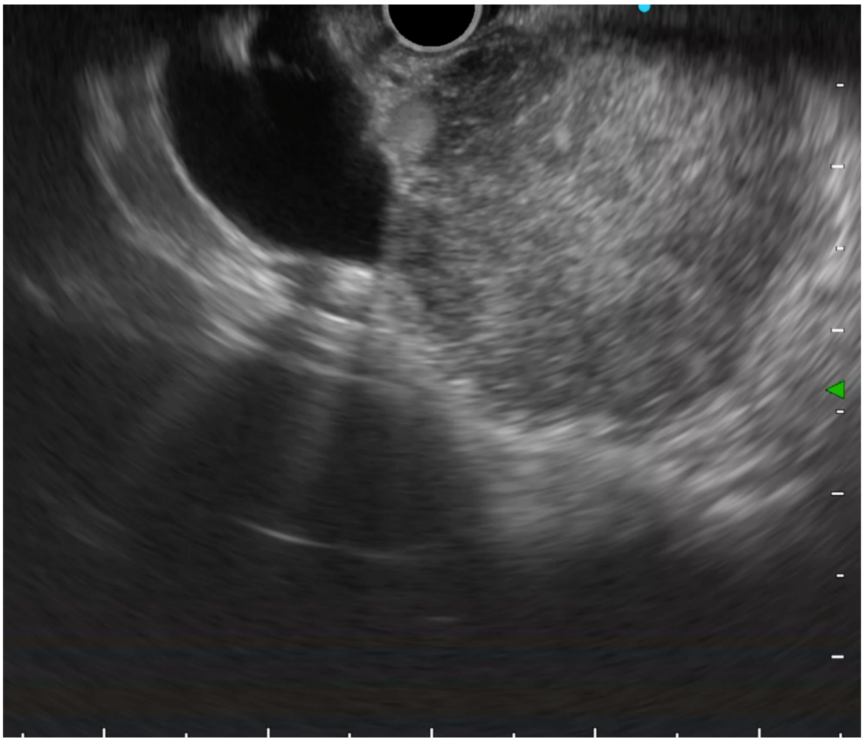

Figure 3. Distal common bile duct obstruction from pancreatic mass.

Ancillary techniques for IBS evaluation: cholangioscopy

The idea of a direct endoscopic visualization of the biliary tree ("cholangioscopy") emerged since the beginning of the ERCP era, in the late 70's,[23] but the need of two operators with high expertise, the limited range of movement, the costs and fragility of the equipment have limited the dissemination of this method.
The development of single-operator cholangioscopy (SOC) and the evolution to digital cholangioscopy (DSOC) overcame these limitations, and nowadays DSOC is largely used in referral centers for both diagnostic and therapeutic purposes.[24,25]
The most used cholangioscope (SpyGlass Discover™, Boston scientific, MA,USA) has a diameter of 3.5 mm (10.5 French) with a four-way steering tip, an operative channel of 1.2 mm (which enables to insert several device for both diagnostic and therapeutic purposes) and two irrigation channels of 0.6 mm; it is usually inserted in the operative channel of a standard duodenoscope and driven inside the biliary tree over a guidewire.

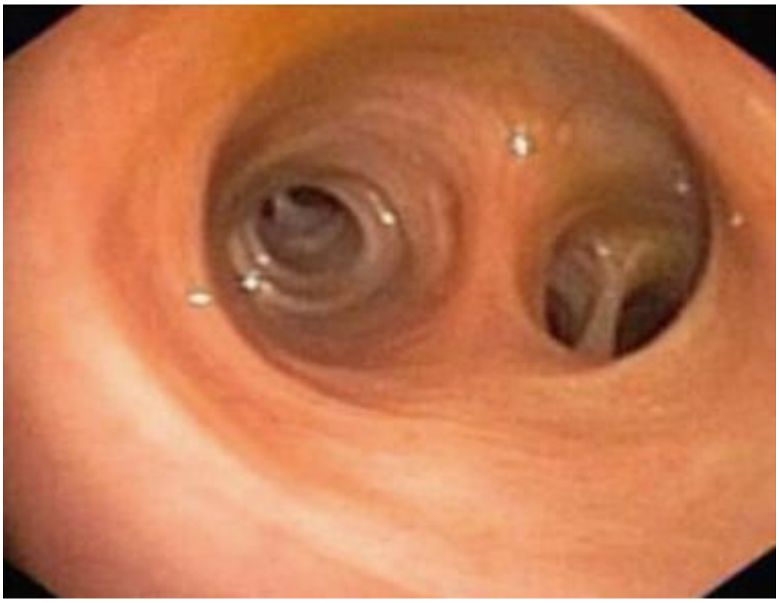

Figure 4. Normal appearance of biliary mucosa during cholangioscopy.

Due to the possibility of a direct visualization of the IBS and targeting biopsies, cholangioscopy has a recognized role in the evaluation of IBS; prospective studies have shown a sensitivity range of 83-97% for DSOC, and a specificity range of 89-93% for digital cholangioscopy.[26–28] A recent meta-analysis of six studies and more than 280 patients using DSOC found a sensitivity of 94% and specificity of 95% in the diagnosis of malignant strictures based on visual interpretation.[29]

Various morphological characteristics have been proposed to predict malignancy during DSOC, such as the presence of papillary projections, intraductal nodules, asymmetrical stricture with ulcerations or infiltrative appearance, dilated and tortuous vessels;[9] however, the interobserver agreement between expert endoscopists has been shown to be slight, demonstrating the need for formally established and validated visual criteria both for benign and malignant stricture.[30]

In 2020, the Monaco classification was developed to create a standardized system of differentiation for malignant and benign lesions. Eight characteristics were included: presence of a stricture (asymmetric or symmetric), presence of a lesion, mucosal features (smooth or granular), papillary projections, ulcerations, abnormal vessels, scarring (local or diffuse), and pronounced pit pattern. The diagnostic accuracy of the Monaco classification was 70%, with an increased interobserver agreement compared to previous published paper, and finding ulcerations and papillary projections being the most highly associated with malignancy.[31]

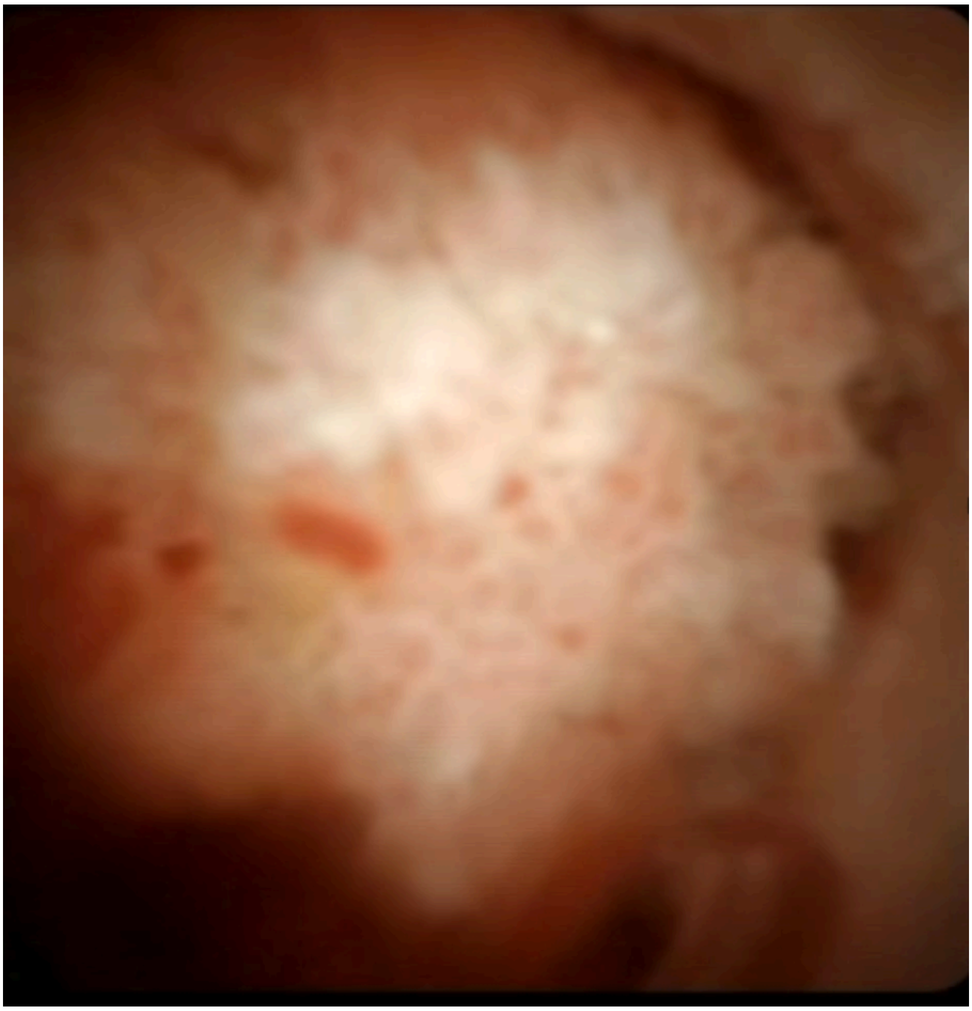

Figure 5. An intraductal polypoid lesion visualized during DSOC.

Regarding tissue acquisition during DSOC, targeted biopsies have demonstrated to be superior to standard tissue acquisition with brushing during ERCP;[32] despite that, the sensitivity is still not ideal and seems to be inferior to DSOC visualization. Several factors have been implicated in suboptimal sampling of bile duct malignancies, including desmoplastic reactions with low cellularity, submucosal spread, ulcerations and fibrosis.[33]

Therefore, other diagnostic modalities able to “look beyond” the mucosal surface of biliary duct may have the potentiality to increase diagnostic accuracy.

Ancillary techniques for IBS evaluation: intraductal ultrasound

Intraductal ultrasound (IDUS) can obtain real-time, cross-sectional images of ductal and periductal structures using a high-frequency ultrasound probe, inserted directly into the bile duct over a guidewire. Findings consistent with a malignant etiology include asymmetric wall thickening, disruption of layers, enlarged lymph nodes and hypoechoic sessile masses or nodules;[34] findings associated with benign strictures include normal layering, smooth margins, and homogenous echo patterns.[35]
In the largest, retrospective study on almost 400 patients, IDUS had a sensitivity of 93.2%, specificity of 89.5% and a diagnostic accuracy of 91.4% in the evaluation of IBS;[36] moreover, a combination of ERCP and IDUS improved diagnostic accuracy from 76% to 88% compared to ERCP alone.[37]
Despite promising data from literature, the utilization of IDUS remains limited by the need of a tissue diagnosis (especially in patients requiring neo-adjuvant treatments) and by the specific training required for images interpretation.[1]

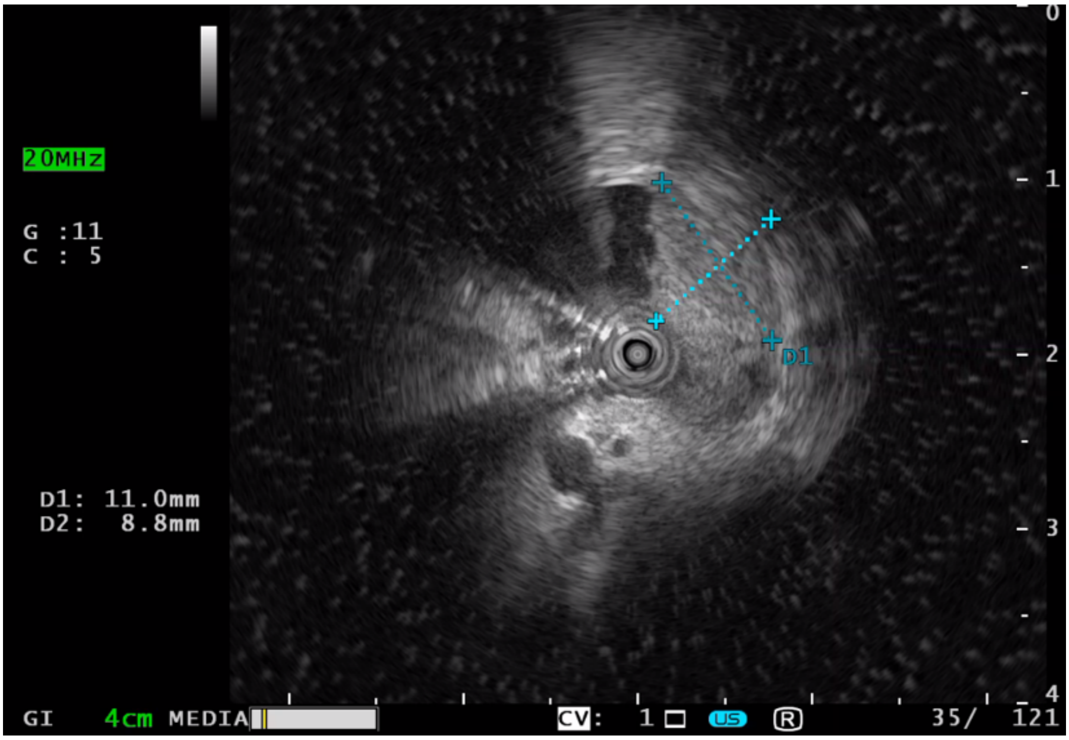

Figure 6. Intraductal malignant mass at IDUS.

Ancillary techniques for IBS evaluation: probe-based confocal laser endomicroscopy

Probe-based confocal laser endomicroscopy (pCLE) uses a low-power laser to provide real time *in vivo* high resolution and magnified images of the mucosal layer; a contrast agent (usually fluorescein) is injected intravenously to highlight the vascular structures while a dedicated probe (CholangioFlex^{TM}, Mauna Kea Technologies, Paris, France) is advanced into the biliary system to capture a "optical biopsy". The probe has a diameter of 0.94 mm, a field of view of 325 μm, a lateral resolution of 3.5 μm, and provides images from 40 to 70 μm below the tissue surface.[38] In the last decades, several applications have been described both in gastrointestinal disorders (i.e. Barrett esophagus, inflammatory bowel diseases, pancreatic cystic lesions) and non-gastroenterological setting (i.e. bladder cancer, lung nodules).[39–41]

Regarding the use of pCLE in the evaluation of IBS, two classifications have been developed to differentiate malignant from benign strictures. In the Miami classification, the presence of thick white bands (> 20 μm), thick dark bands (> 40 μm), loss of reticular pattern of epithelial bands and dark clumps can predict malignancy with a sensitivity of 97% and a specificity of 33%.[42] The lack of specificity was probably due to a partial overlap between findings of malignant and inflammatory strictures, leading to a high false positive rate; as a result, the Paris inflammatory criteria were developed. These categorize multiple white bands, dark granular pattern in scales, enlarged space between scales, and thickened reticulum as consistent with inflammation.[43] Combining the ERCP impression with pCLE using the Miami and Paris classifications, Slivka and colleagues demonstrated a sensitivity of 89%, a specificity of 71% and an overall diagnostic accuracy of 82%, which increased to 88% when also histopathology was added to global evaluation of the patient.[44] Similarly, a meta-analysis of 8 studies showed a pooled sensitivity of 90% and pooled specificity of 72%.[45] However, it is to be noted that in the majority of the studies included in the above mentioned meta-analysis, pCLE was delivered mostly through a catheter during ERCP under fluoroscopic view; this means that the "optical biopsy" was performed without a direct visualization of the stricture, which can help to target suspicious areas of an IBS.

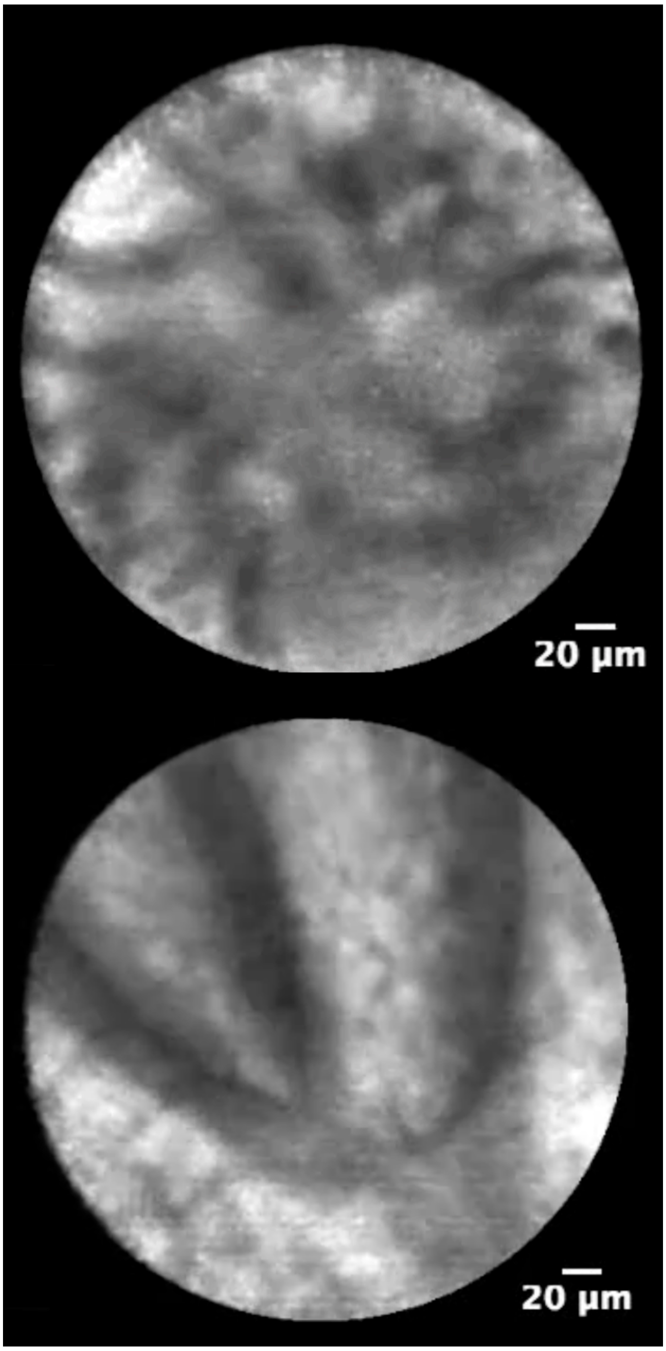

Figure 7. pCLE of an inflammatory biliary stricture (upper) and of a malignant stricture (lower).

EMERGING TECHNIQUES FOR IBS EVALUATION

Several new diagnostic techniques are emerging as promising tool in the evaluation of IBS; these include intraductal optical coherence tomography (OCT) and the molecular diagnostic with next generation sequencing (NGS) and fluorescent in situ hybridization (FISH); despite encouraging data, lack of standardization and costs have limited the diffusion of these technologies.[46–48]

AIMS OF THE STUDY

Due to the low sensitivity of standard techniques, a “one shot” approach may be offered in some referral centers, with a combination of EUS with ERCP and ancillary techniques; this is generally the approach in our Endoscopy Unit at AOU Città della Salute e della Scienza di Torino (Turin, Italy), with the application of the “EURCP” concept.[49] In order to maximize the diagnostic yield, we provide a tailored approach with the utilization of one or more ancillary techniques (cholangioscopy and targeted biopsy under cholangioscopic view, IDUS, pCLE), trying to reduce the need for multiple procedures.

Aim of this study was to evaluate diagnostic performance (sensitivity, specificity, accuracy, positive and negative predictive values) of DSOC, DSOC-guided biopsy, IDUS, pCLE and standard brush cytology in patients with IBS. Secondary aims were the evaluation of the safety profile of these techniques, and the evaluation of the impact of a previous stenting on diagnostic accuracy.

PATIENTS AND METHODS

STUDY DESIGN

This is a monocentric, prospective, observational study on patient with IBS underwent cholangioscopy at the Endoscopy Unit of AOU Città della Salute e della Scienza di Torino (Turin, Italy), from January 2018 to September 2022.

STUDY POPULATION

This study included patients with a previous inconclusive diagnostic work up for biliary stricture (with ERCP and brushing cytology and/or EUS with FNA/FNB); patients could be referred from other hospitals, or have had the previous procedures at our Unit.

All demographic, clinical, endoscopic, histologic and follow-up data were collected prospectively in an electronic database.

Inclusion criteria:

- Patients with IBS underwent DSOC for IBS between January 2018 and September 2022
- Patients $\geq$ 18 years of age

Exclusion criteria:

- Indication to DSOC other than IBS
- Coagulation disorder with a contraindication to invasive endoscopic maneuvers (INR >1.6, platelet count < $40x10^3/mm^3$)
- Refusal to give informed consent to the procedure and/or to the study
- Known allergy to fluorescein or contraindication to fluorescein injection (beta blocker therapy and arrhythmia).[50]

PROCEDURE

All procedures were performed by highly experienced biliopancreatic endoscopists and prior training and experience for all the ancillary techniques (pCLE, IDUS and cholangioscopy); endoscopists were not blinded to relevant clinical information before the procedures. ERCP was performed in standard fashion with a TJF-180 duodenoscope (Olympus, Tokyo, Japan) with Propofol-induced deep sedation and patient in left lateral decubitus; antibiotic prophylaxis was administered to all patient before cholangioscopy (generally beta-lactam or fluoroquinolones) and rectal

indomethacin or diclofenac (100 mg) were administered in all patients for post-ERCP pancreatitis prophylaxis, if not contraindicated. Patients stayed at least one night in hospital after procedure and blood tests, including lipases, were performed 6 hour after the procedure and the next morning.
IDUS examination was carried out with the introduction of a 20 MHz wire-guided miniprobe (UM-DP20-25R, Olympus, Tokyo, Japan) above the stricture, and then gently passed through the stricture for the evaluation of ultrasonographic images.
Cholangioscope for DSOC (SpyGlass Discover™, Boston scientific, MA,USA) was introduced over a guidewire up to the stricture, for a visual inspection.
After visual inspection, pCLE was performed before manipulating the stricture with biopsies or brushing, to avoid artifacts; upon administration of 2.5 mL of 10% fluorescein intravenously, image collection began with video recording of pCLE examinations using a dedicated probe (CholangioFlex™, Mauna Kea Technology, Paris, France) advanced through the working channel of the cholangioscope. The pCLE probe was positioned as perpendicular as possible in direct contact with the biliary mucosa and the video was examined real-time and recorded for further evaluation, if needed.
DSOC-guided biopsies were performed with dedicated forceps (SpyBite™, Boston Scientific; MA, USA) targeting suspicious areas of the IBS.
Brushing cytology was performed under fluoroscopic view, with a standard brush (Cytomax II Double-lumen Cytology Brush, Cook Medical, NC, USA).
DSOC and DSOC-guided biopsy were planned in all procedures; the decision to perform pCLE, IDUS, and/or brushing cytology was left to the discretion of the endoscopist based on clinical or anatomical evaluation.

DEFINITIONS

For DSOC-based diagnosis, the presence of the following criteria was evaluated: (1) stricture, (3) mucosal changes, (4) papillary projections, (5) ulceration, (6) mass or nodularity, and (7) vascularization;[51] the final impression diagnosis of benign or malignant stricture was made during the procedure.
For pCLE-based diagnosis, Miami classification and Paris classification were used. For the Miami Classification, 2 or more criteria of the following were required for a malignant impression: (1) thick, dark bands >40 µm, (2) thick, white bands >20 µm, (3) dark clumps, and (4) epithelial structures. The Paris Classification was used to describe inflammatory characteristics of biliary strictures and included the following: (1) vascular congestion, (2) dark granular patterns with scales, (3) increased

interglandular space, and (4) thickened reticular structures. [42,43]All pCLE impressions were made real-time during the ERCP and reviewed after the procedure, if needed.
IDUS-based diagnosis was made on the basis of previous published criteria: asymmetric wall thickening, disruption of layers, enlarged lymph nodes and/or hypoechoic sessile masses or nodules were considered consistent with malignant stricture.[34]
As reference diagnosis of malignancy, surgical specimen (when available), biopsy and/or cytology showing malignant cells were considered**;** a benign diagnosis required a minimum of 6 months of clinical and radiological follow-up with no masses or evolution seen on imaging, repeated sampling or death.
The length of follow up was calculated as the time between procedure and surgery in patients underwent surgical resection; for other patients, the time between procedure and death or the last clinical contact was calculated.
Adverse events were investigated with scheduled visits, phone calls and/or in electronic health records, and categorized by the onset (preprocedural, intraprocedural, post-procedural as <14 days and late as ≥ 14 days) and the severity (mild, moderate, severe and fatal).[52]

Statistical analysis

Demographic, clinical, procedural and pathology details were depicted using descriptive statistic, as mean and standard deviation (±SD) for continuous variables, or number and percentage for categorical variables.
Operating characteristics including sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were calculated for each diagnostic technique.
Fisher's exact test was applied for the comparison of the operating characteristics, Student's t-test was used for comparison of continuous variables among subgroups.
P value of 0.05 was considered to be statistically significant. Statistical analysis was performed with MedCalc Statistical Software version 19.2.6 (MedCalc Software bv, Ostend, Belgium) and GraphPad Prism version 8.0.0 (GraphPad Software, CA, USA).

RESULTS

A total of 57 patients were evaluated, mostly male (39 patients, 68.4%) with a mean age of 67.2 ± 10.0 years. The majority of patients had comorbidities (34 patients, 59.6%), particularly cardiovascular diseases (24 patients, 42.1%), chronic liver diseases (7 patients, 12.3%) and pulmonary diseases (6 patients, 10.5%).
Only one patient had a previous diagnosis of primary sclerosing cholangitis, and 11 patients (19.3%) had a history of previous or active tobacco consumption.
All patients had a previous inconclusive diagnostic procedure: 39 patients had previous ERCP (68.4%), with a mean number of previous procedures of 1.97 ± 1.42; 14 patients had a previous attempt of IBS characterization with EUS ± FNA/FNB (24.6%) and 4 patients (7.0%) had a previous attempt with percutaneous ultrasound transhepatic biliary tissue acquisition; 38 patients (66.7%) had a previous biliary sphincterotomy and 30 patients (52.6%) had a biliary stent in place.
Strictures were located along the whole biliary tree; the most common location of IBS in our cohort was the distal common bile duct (26 patients, 45.6%), followed by the common hepatic duct (13 patients, 22.8%), hepatic hilum (12 patients, 21.1%), intrahepatic ducts (5 patients, 8.8%) and the cystic duct (1 patient, 1.7%). Baseline characteristics of patients are reported in table 2.
DSOC was successfully performed in all patient; 52 patients underwent DSOC-guided tissue acquisition. In 5 patients, DSOC-guided biopsy was not performed (in 2 patients because of the evidence of extra-ductal lesion, in 3 patients for technical failure in bringing the forceps out from the cholangioscope); the technical success rate of DSOC-guided biopsy was then 94.5%
Fifty-two patients underwent IDUS: the passage of the miniprobe through the stricture was not possible in 3 patients, while in the other 2 cases IDUS was temporary unavailable. The technical success rate of IDUS was 94.5%. Lastly, 27 patients underwent pCLE and 39 brush cytology.
Final diagnosis was consistent with malignancy in 35 patients (61.4%), and cholangiocarcinoma was the most common etiology (77.1%), followed by intraductal papillary biliary neoplasm with high grade dysplasia (4 patients, 11.4%), ampullary adenocarcinoma (3 patients, 8.6%) and pancreatic cancer (1 patient, 2.9%). The final diagnosis of malignancy was confirmed by surgical pathology in 22 patients (62.9%), by evidence of malignancy in cytology and/or DSOC-guided biopsies in 9 patients (25.7%) and by clinical and radiological follow up in 4 patients (11.4%).
A benign etiology of IBS was found in 22 patients, confirmed by surgical pathology in one patient and by clinical and radiological follow up in 21 of them. Final diagnosis are resumed in Figure 8.

The mean follow-up of patients was 18.2 ± 18.1 months; in patient with a final diagnosis of benign strictures was 24.1 ± 18.6 months, while the mean follow-up of patients with malignant strictures was 14.5 ±17.1 months.

Table 2. Baseline characteristics of patients

Mean age (±SD), years	67.2 (±10.0)
Male/Female, n (%) / n (%)	39 (68.4%) / 18 (31.6%)
Comorbidities, n (%)	34 (59.6%)
Cardiovascular	24 (42.1%)
Pulmonary	6 (10.5%)
Liver diseases	7 (12.3%)
Chronic kidney disease	1 (1.7%)
Type II diabetes mellitus	10 (17.5%)
Tobacco consumption	11 (19.3%)
Patients with previous ERCP, n %	39 (68.4%)
Mean previous ERCP (±SD)	1.97 ± 1.42
Patients with stent in place prior to DSOC, n (%)	29 (50.9%)
Previous biliary sphincterotomy, n (%)	38 (66.7%)
Previous cholecystectomy, n (%)	21 (36.8%)
Stricture location, n (%)	
Common bile duct	26 (45.6%)
Common hepatic duct	13 (22.8%)
Cystic duct	1 (1.7%)
Hepatic hilum	12 (21.1%)
Intrahepatic ducts	5 (8.8%)

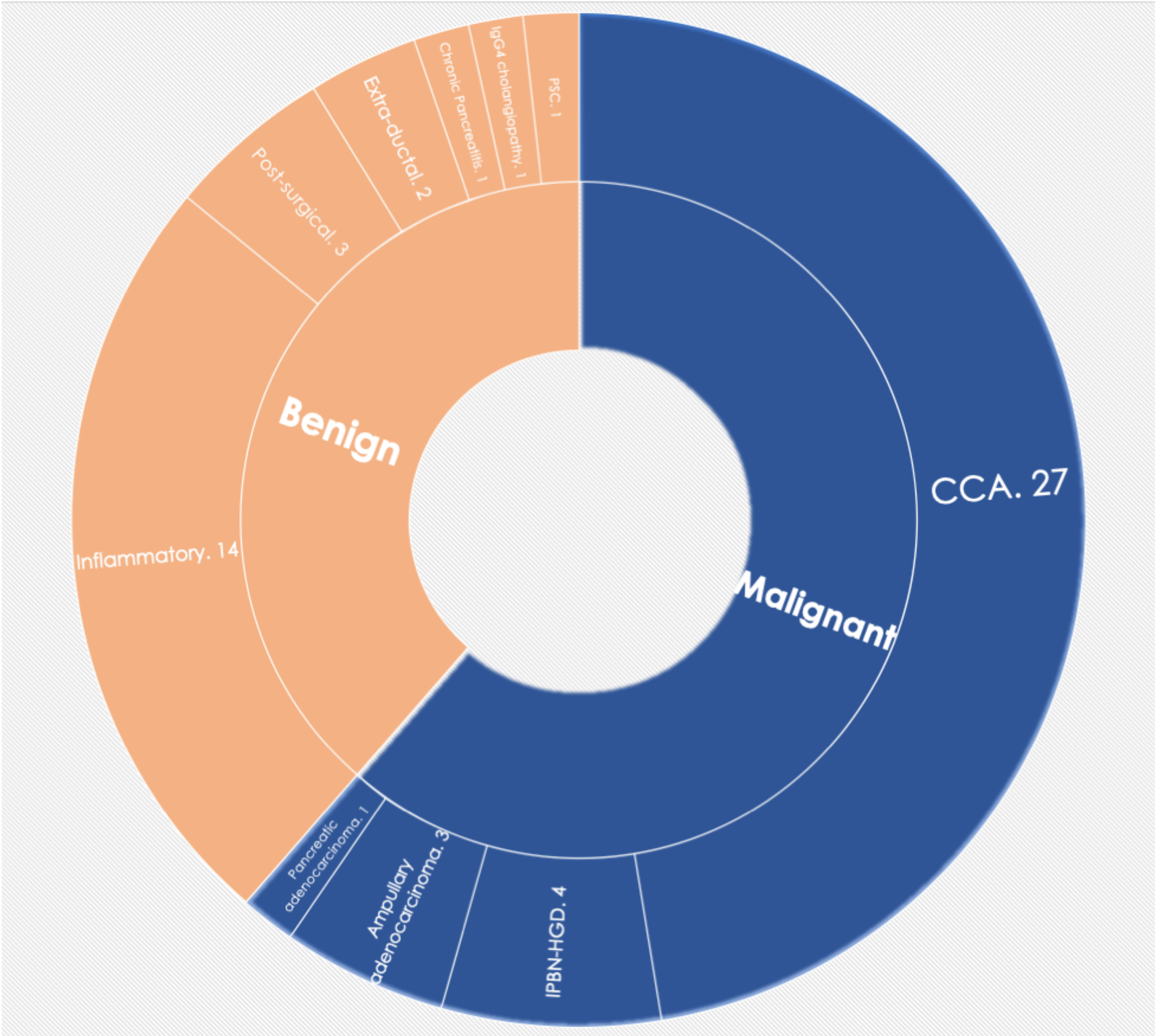

Figure 8. Final diagnosis in the cohort of patients

DIAGNOSTIC PERFORMANCE

Diagnostic yields of different techniques are shown in table 3.
DSOC showed a sensitivity of 85.7% (CI 95% 76.6 – 94.8%), a specificity of 95.5% (CI 95% 90 – 100%) and an overall diagnostic accuracy of 89.5% (CI 95% 81.5 – 97.4%); NPV was 80.8% (CI 95% 70.5% - 91%) and PPV was 96.8% (CI 95% 92.2.- 100%).
pCLE had a sensitivity of 84.2% (CI 95% 70.8 – 97.6%), a specificity of 87.5% (CI 95% 71.1 – 100%) and an accuracy of 85.2% (CI 95% 71.8 – 98.6%). NPV and PPV were respectively 70% (CI 95% 56.6% - 83.4%) and 94.1% (CI 95% 80.7 – 100%).

IDUS showed similar characteristics, with a sensitivity of 84.4% (CI 95% 74.5 – 94.2%), a of 80% (CI 95% 69.1 – 90.9%) and an accuracy of 82.7% (CI 95% 72.4 – 93%). NPV and PPV were respectively 76.2% (64.6% - 87.8%) and 87.1% (CI 95% 78 – 96.2%).
DSOC-guided biopsies had lower sensitivity (63.6%, CI 95% 51.1% - 76.1%) and a specificity of 100% (83 – 100%); the diagnostic accuracy of targeted biopsies was 76.9% (CI 95% 66 – 87.9%).
Brush cytology showed a sensitivity of 51.6% (CI 95%38.6 – 64.6%) and a specificity of 100% (CI 95% 68 – 100%). The accuracy of this technique was 61.5% (CI 95% 48.9 – 74.2%).

Table 3. Diagnostic yield of different techniques

Techniques	Sensitivity (CI 95%)	Specificity (CI 95%)	Accuracy (CI 95%)	NPV (CI 95%)	PPV (CI 95%)
DSOC visualization	85.7% (76.6 – 94.8%)	95.5% (90.0 – 100%)	89.5% (81.5 – 97.4%)	80.8% (70.5 – 91%)	96.8% (92.2 – 100%)
pCLE	84.2% (70.8 – 97.6%)	87.5% (74.1 – 100%)	85.2% (71.8 – 98.6%)	70.0 % (56.6 – 83.4%)	94.1% (80.7 – 100%)
IDUS	84.4% (74.5 – 94.2%)	80.0% (69.1 – 90.9%)	82.7% (72.4 – 93.0%)	76.2% (64.6 – 87.8%)	87.1% (78.0 – 96.2%)
DSOC targeted biopsy	63.6% (51.1 – 76.1%)	100% (83.0 – 100%)	76.9% (66.0 – 87.9%)	61.3% (48.6 – 73.9%)	100% (85.0 – 100%)
Brush cytology	51.6% (38.6 – 64.6%)	100% (78 – 100%)	61.5% (48.9 – 74.2%)	34.8% (22.4 – 47.1%)	100% (82 – 100%)

DSOC visualization, pCLE and IDUS outperformed cytology in terms of sensitivity ($p< 0.01$, $p= 0.03$, $p< 0.01$ respectively) and accuracy ($p< 0.01$, $p= 0.047$, $p= 0.03$ respectively); NPV was significantly higher for DSOC and IDUS compared to cytology ($p< 0.01$). Finally, DSOC sensitivity was significantly higher than targeted biopsies ($p= 0.05$).
Comparison of operating characteristics of each test are reported in table 4.

Table 4. Comparison among diagnostic techniques.

	Sensitivity	**Specificity**	**Accuracy**	**NPV**	**PPV**
Comparison	**P value**				
DSOC vs pCLE	>0.99	0.47	0.72	0.66	>0.99
DSOC vs IDUS	>0.99	0.17	0.41	0.73	0.35
DSOC vs Biopsy	**0.05**	>0.99	0.12	0.15	>0.99
DSOC vs Cytology	**<0.01**	>0.99	**<0.01**	**<0.01**	>0.99
pCLE vs IDUS	>0.99	>0.99	>0.99	>0.99	>0.99
pCLE vs Biopsy	0.48	0.30	0.56	0.66	0.45
pCLE vs Cytology	**0.03**	>0.99	**0.047**	0.12	0.57
IDUS vs Biopsy	0.09	0.11	0.63	0.73	0.11
IDUS vs Cytology	**<0.01**	0.29	**0.03**	**<0.01**	0.23
Biopsy vs Cytology	0.45	>0.99	0.16	0.10	>0.99

SECONDARY AIM: EFFECT OF PREVIOUS STENTING

Twenty-nine out 57 patients (50.9%) underwent the procedure with a previous stent in place, removed before starting DSOC, and all but one were plastic stents. Twenty-eight patients (49.1%) did not have stent in place.
The two groups were comparable in terms of age (respectively 66.5 ± 11.0 vs 68.0 vs 9.2, $p = 0.58$), gender (male were 62.1% and 75% respectively, $p = 0.27$) and etiology (malignant stricture in 55.2% and 67.9% of cases, $p = 0.42$).
Presence of a stent did not affect the diagnostic accuracy of DSOC-visualization (89.7% in the stent group, 92.9% in the no-stent group, $p >0.99$); the diagnostic accuracy of IDUS and pCLE in stented patients had a slight decrease (respectively 74.1% vs 92% and 71.4% vs 100%) but the reduction was not statistically significant ($p = 0.14$ and $p = 0.10$, respectively).

SECONDARY AIM: SAFETY

Ten patients out of 57 experienced 11 adverse events (17.5%); 5 adverse events were mild, 5 moderate and 1 fatal.

The most common adverse event was cholangitis (5 cases, 8.8%), followed by acute pancreatitis (4 cases, 7.0%) and one case of aspiration pneumonia; only in one case of cholangitis a new endoscopic intervention was required, with replacement of malfunctioning plastic stent, while the others were managed with medical therapy. In all cases the hospital stay was prolonged for less than 7 days.

A patient with a previous history of coronary artery disease developed a myocardial infarction four days after cholangioscopy, which was judged not related to endoscopic procedure; he underwent to percutaneous transluminal coronary angioplasty and died during the hospitalization.

All adverse events are reported in table 5.

Table 5. Adverse events

Patient	Adverse event	Severity grade	Onset (day after procedure)	Management	Outcome
Patient 1	Cholangitis	Moderate	9	Endoscopic (repeated ERCP with stent replacement)	Favorable
Patient 2	Acute pancreatitis	Mild	1	Medical therapy	Favorable
Patient 2	Cholangitis	Moderate	2	Medical therapy	Favorable
Patient 3	Acute pancreatitis	Mild	0	Medical therapy	Favorable
Patient 4	Acute pancreatitis	Mild	0	Medical therapy	Favorable
Patient 5	Myocardial infarction	Fatal	4	Percutaneous transluminal coronary angioplasty	Fatal
Patient 6	Aspiration pneumonia	Moderate	0	Medical therapy	Favorable
Patient 7	Cholangitis	Moderate	1	Medical therapy	Favorable
Patient 8	Cholangitis	Mild	1	Medical therapy	Favorable
Patient 9	Cholangitis	Mild	1	Medical therapy	Favorable
Patient 10	Acute pancreatitis	Moderate	0	Medical therapy	Favorable

DISCUSSION

The goal of an accurate diagnosis of indeterminate biliary strictures is crucial due to the potentially vastly different prognosis based on etiology.[3] Because of the suboptimal diagnostic accuracy of conventional ERCP-based tissue acquisition, DSOC has emerged as a promising innovation that could have a role in the diagnosis of IBS.[24] Our results confirm these findings: DSOC, pCLE and IDUS showed high diagnostic accuracy (respectively 89.5%, 85.2% and 82.7%), significantly higher when compared with brush cytology (61.5%) which is a current standard in many endoscopy units.

The direct visualization of the characteristics of a stricture results in the possibility to recognize endoscopic features of malignancy, such as papillary projections and tortuous vessels, with a specificity comparable with histological specimen but a higher sensitivity.

Interestingly, an optical diagnosis seems to perform better in term of sensitivity and diagnostic accuracy compared to DSOC-guided biopsies. This "paradox" can be partially explained by biological characteristics of cholangiocarcinoma, which is the leading cause of IBS in our cohort. Tumor associated fibrosis or ulceration is a recognized finding in mucosal malignancies of the gastrointestinal wall and can affect the ability to obtain adequate cellularity. Desmoplastic tumors are relatively firm and have lower cellularity, making sampling difficult; moreover, some tumors of the bile duct exhibit submucosal spread, which will particularly lower the yield of superficial sampling method. Lastly, cancers extrinsic to the bile duct such as pancreatic cancers and metastatic tumors are expectedly more difficult to sample from within the duct.[33]

In addition, size and shape of the DSOC-dedicated forceps may be implicated in the lack of sensitivity of targeted biopsy; as previously described,[53,54] the small specimens collected by the forceps have to be carefully manipulated to avoid loss of material during the standard formalin fixation and paraffin embedding, and new dedicated processing protocols should be individuated in order to maximize the diagnostic yield.[55]

Unlike in EUS guided tissue acquisition,[56] the procedure is still lacking of a standardized protocol of sampling, and the minimum number of biopsies to optimize the diagnostic performance of the technique has not been defined. However, in a prospective study, Bang and colleagues found that in the absence of on-site cytopathology evaluation, performing 3 biopsies can make the correct diagnosis for the 90% of cases, comparable to the on-site approach.[57] Indeed, when available, the "rapid on-site evaluation of touch imprint cytology"(ROSE-TIC) may improve the diagnostic yield of DSOC-guided biopsies.[58]

Our results are in line with literature, where the visual accuracy during DSOC has been reported to range between 80 and 97%.[27,59] Initially, some concerns were raised on the poor interobserver agreement for the correct classification of some cholangioscopic features,[60] based more on impressions provided by the investigators rather than reference to standardized, validated definitions.[61,62]

The evolution of cholangioscopy to a digital platform with improved imaging quality[63] and the introduction of different classification systems (the Monaco classification and the Robles-Medranda Criteria)[31,64] helped to overcome these limitations, as demonstrated recently by Kahaleh and colleagues.[65] In this paper, authors find an improved interobserver agreement (IA) for items such as lesions (0.75) and finger-like papillary projections (0.74), and good IA for tortuous vessels, mucosal features, uniform papillary projections, and ulceration (0.53–0.7), between 15 interventional endoscopist with high expertise in DSOC.

Our study shows a similar diagnostic yield for DSOC and ancillary techniques such as pCLE and IDUS, which is significantly higher compared to standard cytology; to our knowledge, this is the first study showing a direct comparison in the same set of patient of all these techniques.

Interpretation of pCLE imaging can be challenging and needs adequate training; a recent meta-analysis of 8 studies showed a pooled sensitivity of 90%, comparable to our study, but a specificity of 70%, lower compared to our results;[45] a partial explanation can be found in the way of pCLE delivery. In our experience, the probe was placed on the stricture under direct visualization, while several previous published study included both patient with “cholangioscopy assisted” pCLE and through-the-catheter pCLE, under fluoroscopic-guidance; in this setting, targeting the lesion can be difficult and a suboptimal scanning plan can affect the quality of pCLE images. Nevertheless, the ability to perform pCLE through a catheter independently from DSOC is undoubtedly an advantage: distal stricture and tight angulation can reduce the technical success rate of DSOC, due to the intrinsic struggle to introduce and advance the cholangioscope.[66]

The present study showed similar diagnostic yield for IDUS, with sensitivity and specificity above 80%, significantly higher compared to cytology; IDUS showed also a trend for a better sensitivity compared to targeted biopsies (84.4% vs 63.6%, $p = 0.09$). This technique has not proliferated, and nowadays very few endoscopists performing ERCP are trained in IDUS. In our experience, it is a fast and reliable tool in the evaluation stricture, residual lithiasis and compression of the bile ducts from extra-ductal lesions, which can be hard to assess by fluoroscopy; moreover, it permits an evaluation of longitudinal extension of cholangiocarcinoma, and provides an accurate assessment of hepatic artery and portal vein infiltration, which is crucial for surgical candidates.[67] Not least, compared to cholangioscopy, IDUS is safer and less expensive. In fact, miniprobes are reusable and can last up to 100 examinations when properly handled, which made IDUS particularly beneficial in limited-resource settings.[68]

An interesting finding of our study is the impact of biliary stenting on diagnostic accuracy. Diagnostic accuracy of DSOC with or without a stent in place is comparable (respectively 89.7 vs 92.9), while diagnostic accuracy of pCLE and IDUS in patient with stent in place was slightly decreased. In fact, pCLE in stented patient had an accuracy of 71.4% (compared to 100% of accuracy in patient without stent), and something similar was observed for IDUS (74.1% of accuracy in the stent group, compared to 92% in patient without biliary stenting).

These differences, although not significant, show how prior endoscopic manipulation of biliary stricture alters the biliary epithelium, likely resulting in inflammation and architectural distortion which can negatively impact accuracy.[69] The application of specific, previous published pCLE criteria for inflammatory strictures[70] permitted to mitigate the loss in specificity (and overall accuracy) described in previous papers.[42]

In addition to the diagnostic yield of different techniques, we focused on safety of procedure; adverse events rate of our study was 17.5%, which is comprised in the wide range (from 1.7% to 25.4%) published in previous studies;[63,66,71] this variation depends to a certain extent to different definition of adverse events in previous published study, with higher rates in papers with more detailed definitions. Despite the administration of antibiotic prophylaxis in all patients, the most common adverse event was cholangitis (8.8%), probably due to the intermittent irrigation to obtain adequate visualization of the biliary mucosa, with a retrograde bacterial flow in the biliary tree.[72]

Currently, European guidelines consider cholangioscopy as a procedure at high risk for post-ERCP cholangitis and suggest antibiotic prophylaxis when performing it.[73] With this in mind, more studies, including randomized controlled trials, are necessary to precisely define possible measure to prevent this complication, maybe investigating a possible role of a longer course of antibiotic therapy after cholangioscopy, instead of only procedural antibiotic dose, in reducing the rate of cholangitis rate.

Acute pancreatitis was the second most common adverse in our study , occurring in 7% of patients. This rate seems greater when compared to the 2-4% risk of acute pancreatitis following standard ERCP.[74]

A possible pathogenic mechanism can be found in the mechanical irritation and subsequent swelling of the papilla due to the passage of the cholangioscope, as previously described for other rigid catheter such as IDUS miniprobes.[75] Interestingly, the association of the two techniques (DSOC and IDUS) in our cohort did not lead to an increased risk when compared to previous published studies on DSOC.[63,66]

Furthermore, all cases of post-procedural acute pancreatitis observed were mild and moderate, managed with medical therapy and with limited extension of the hospital stay. Since post-procedural lipase levels were routinely determined in all patients, even mild cases of pancreatitis were less likely to be missed.

Before drawing conclusions, we need to address some limitations to our study. First of all, endoscopists were not blinded to the previous imaging, laboratory results and clinical history when they were evaluating biliary strictures, hence IDUS, pCLE and DSOC visual evaluation could have been biased; however, our patients were referred due to still indeterminate biliary strictures despite previously performed diagnostics, and this is more representative of the clinical practice. Secondly, this is a monocentric experience, with procedures performed by expert endoscopists in a high volume referral hospital; although European guidelines recommend that indeterminate strictures should be assessed and managed in tertiary referral centers,[76,77] it is unclear whether similar results would be reproduced if less-experienced endoscopists performed these procedures. Furthermore, in this prospective series the choice to perform each diagnostic techniques was left to the discretion of the endoscopist, and some patients underwent only to a part of the described techniques; however, to our knowledge this is the first study with a direct comparison of four advanced diagnostic techniques (pCLE, IDUS, DSOC visualization and targeted biopsies) with standard cytology in the same set of patients. Lastly, the number of patients (especially those who underwent pCLE) is still relatively small; this explains the wide confidence intervals in our diagnostic operating characteristics and may limit our results given that one false negative or false positive examination can significantly change our findings. Unfortunately, a consistent part of the study was carried out during the COVID-19 pandemic, which had a severe impact on the Italian health care system[78] and on endoscopic units activity.[79,80]

Our hospital and endoscopy unit was involved as well in facing dramatic outbreaks, with the need of massive organizational changes, doctors and nurses reassigned to dedicated treatment of SARS-CoV-2-positive inpatients and the burden of hospitalizations. As described by other groups,[81] we observed a reduction in overall ERCP referrals during the toughest times of pandemic, and this may be for several reasons, such as fear of leaving home, adhering to public health guidance to remain indoors, and reduced availability or access to usual healthcare pathways.

CONCLUSIONS AND FUTURE PERSPECTIVES

DSOC visualization, intraductal ultrasound and probe-based confocal laser endomicroscopy demonstrated an optimal diagnostic yield in the differentiation of indeterminate biliary strictures; the high sensitivity of these techniques compared to standard sampling methods helped to correctly diagnosed 90% of IBS in our cohort. A multimodal approach, with the possibility to perform different diagnostics in the same session with a tailored procedure, can help endoscopists in the management of this challenging disease. DSOC showed the highest diagnostic accuracy, and should be the method of choice in the evaluation of IBS; when technically unfeasible due to the position or angulation of the stricture, the use of pCLE and/or IDUS can come to the rescue, providing a reliable results in more than 80% of the patients and reducing the need of multiple procedures, shortening the time to reach an accurate diagnosis.

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SUMMARY