Role of ERCP for the diagnosis of indeterminate biliary stenosis and the treatment of benign (non-neoplastic) biliary stenosis in adults

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This publication URL: https://archive-ouverte.unige.ch/unige:163015

Publication DOI: 10.13097/archive-ouverte/unige:163015

UNIVERSITÉ DE GENÈVE
FACULTÉ DE MÉDECINE

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ROLE OF ERCP FOR THE DIAGNOSIS OF INDETERMINATE BILIARY STENOSIS AND THE TREATMENT OF BENIGN (NON-NEOPLASTIC) BILIARY STENOSIS IN ADULTS

Thesis submitted to the Faculty of Medicine of
the University of Geneva

for the degree of Privat-Docent
by

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Geneva
2021

ABBREVIATIONS

18F-FDG-PET = 18F-flouro-deoxy-glucose positron emission tomography

cSEMS = covered self-expandable metallic stent

ERCP = endoscopic retrograde cholangiopancreatography

ESGE = European Society of Gastrointestinal Endoscopy

EUS = endoscopic ultrasonography

FCSEMS = fully covered self-expandable metallic stent

FISH = Fluorescence in situ hybridization

FNA = fine-needle aspiration

IUDS = intraductal ultrasonography

MRCP = magnetic resonance cholangiopancreatography

pCLE = probe-based confocal laser endomicroscopy

PCSEMS = partially covered self-expandable metallic stent

PSC = primary sclerosing cholangitis

SOC = single operator cholangioscopy

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1 Summary

In the first part of this thesis, the diagnostic role of Endoscopic Retrograde Cholangiopancreatography (ERCP) in suspected malignant biliary stenosis) is reported. Sophisticated techniques have been proposed to optimize the diagnostic accuracy of this procedure. We report the major contribution of a simple and safe procedure: bile cytological analysis performed during ERCP in combination with cytological brushing illustrated by our experience detailed in 2 publications. The role of ERCP with a single operator cholangioscopy (SOC) for the diagnosis of indeterminate biliary stenosis and its significant impact on patient management is reported in a third publication.

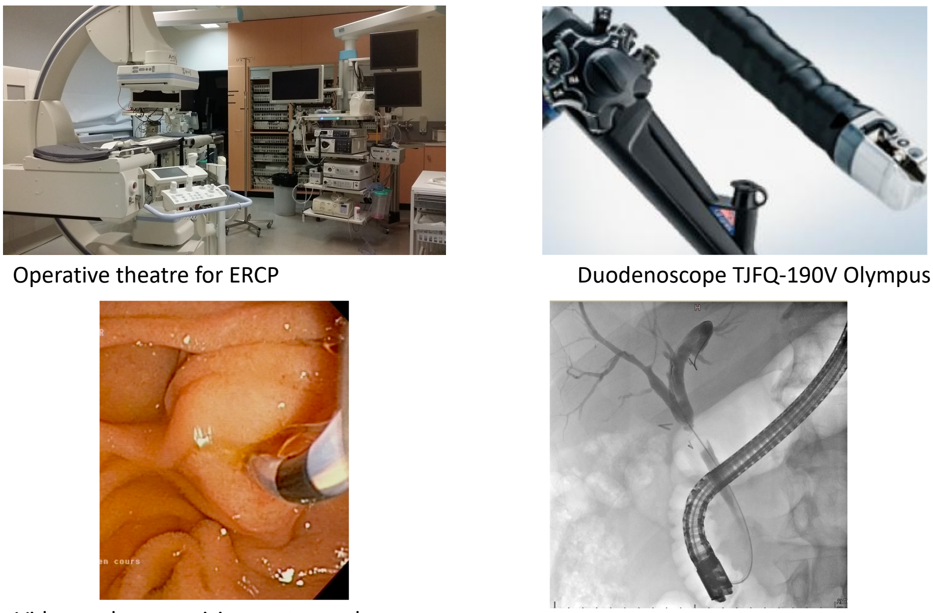
In the second part, the role of therapeutic ERCP is detailed for the treatment of benign biliary stenosis. The role of covered Self-Expandable Metallic Stent (cSEMS) for biliary post-operative stenosis is reported along with our local experience. In addition, ERCP stenting is suggested as an alternative therapy for the particular case of biliary stenosis of alveolar echinococcosis. These two aspects are illustrated by publications.

ERCP remains essential in 2021 for the diagnosis of indeterminate biliary stenosis and the treatment of benign biliary stenosis. In the concluding chapter, we focus on current challenges for teaching ERCP to trainees and standards of performance for practitioners after initial training for this demanding endoscopic technique and research perspectives.

2 Introduction

2.1 ERCP technical aspects and objectives

Endoscopic retrograde cholangiopancreatography (ERCP) is a technique using a specialized side-view endoscope inserted high into the second part of the duodenum to allow access to the major duodenal papilla. The diagnostic and therapeutic devices are introduced into the working channel of the endoscope and then into the bile and / or pancreatic ducts. Opacification by injection of contrast product allows their radiological visualization and the performance of diagnostic and therapeutic procedures (Fig 1).



Operative theatre for ERCP

Duodenoscope TJFQ-190V Olympus

Video endoscopy vision : retrograde catheterism of the major papilla

Fluoroscopic vision : cholangiography (post cholecystectomy main biliary duct stenosis)

Figure 1: Devices for ERCP and imaging (personal material)

ERCP is a complex technique requiring specialized equipment, a long learning curve and the regular practice of a large volume of procedures. Learning ERCP can only be considered after mastering standard gastrointestinal endoscopy.
The benefits of ERCP in the management of biliary and pancreatic pathologies must always be weighed against the risk of serious complications, which is much higher than when performing standard endoscopic techniques [1]. Since the availability of less invasive diagnostic techniques for bilio-pancreatic pathologies, ERCP is only performed in 2021 for diagnostic

purposes in 10% of cases. We report our experience of its practice in the diagnosis of biliary stenosis of undetermined origin; indeterminate biliary stenosis (stricture) and in the treatment of benign (non-neoplastic) biliary stenosis.

2.2 Indeterminate biliary stenosis: place of diagnostic ERCP

2.2.1 Etiologies of biliary stenosis

Large-caliber bile duct stenosis can be of multiple origin, postoperative, post lithiasis, inflammatory; Mirizzi syndrome, primary sclerosing cholangitis, IgG4 related sclerosing cholangitis, ischemic cholangitis, post-radiation cholangitis and most frequently neoplastic; cholangiocarcinoma, extension of gallbladder cancer, hepatocellular carcinoma or locoregional extension of other tumors [2]. Biliary stenosis are benign in 30% of cases[3] and establishing a diagnosis is very important because it is considered that in 3 to 15% of patients, undergoing surgery for a preoperative suspicion of a malignant tumor, the final pathological diagnosis is a benign pathology which may not have required radical surgical therapy. Thus, facing an indeterminate biliary stenosis, the definitive postoperative histopathological diagnosis of which would be the absence of neoplasia, a complex surgical intervention: biliary or hepatic resection or even a Whipple intervention, with a high risk of complications could have been avoided [4] [5] [6].

There is no consensus on the definition of indeterminate biliary stenosis. For most authors, biliary stenosis is considered indeterminate when basic examinations, including imaging exams; US, CT scan, MRCP and ERCP with routine cytological brushing +/- biopsy, do not allow to make a diagnosis [7] [8]. The challenge in 2021 is to increase the diagnostic performance from the first diagnostic ERCP in front of a biliary stenosis whose diagnosis can be considered difficult and to discuss the procedures to be implemented during a second ERCP for the diagnosis of indeterminate biliary stenosis.

2.2.2 Diagnostic approach of biliary stenosis

2.2.2.1 Clinical presentation

The clinical presentation of patients with biliary stenosis is varied. This may be a clinically asymptomatic patient with abnormal liver tests in favor of a biological cholestasis syndrome, mainly with an increase in serum alkaline phosphatase. The onset is gradual, more or less rapid with possible pruritus and then jaundice. Stenosis can be discovered with an acute complication such as cholangitis or a change in general condition. This clinical presentation is common to benign and malignant etiologies.

2.2.2.2 Non-invasive examinations

2.2.2.2.1 Blood tests

Blood tests are of little relevance since there is no biological marker that can distinguish the benign or malignant nature of a biliary stenosis. The 19-9 carbohydrate antigen (CA 19-9) is associated with tumors. This antigen is the same as that of the Lewis blood group It is synthesized by the normal human pancreas as well as by the biliary, gastric, colic, esophageal, endometrial and salivary epithelia. The blood value of the CA 19-9 is not specific, in fact this marker rises in cholestasis: cholangitis, cirrhosis, primary sclerosing cholangitis. However, after biliary drainage, the persistence of a high level of CA 19-9 is in favor of neoplastic biliary stenosis [9].

Elevated IgG4 blood levels, present in more than 70% of IgG4 cholangitis and 10-20% of primary sclerosing cholangitis, may also be present in cholangiocarcinoma or pancreatic cancer. Therefore, a high IgG4 value considered without other criteria does not eliminate the malignant nature of a biliary stenosis [10].

2.2.2.2.2 Abdominal ultrasound (US)

Abdominal US is the first-line examination for suspected obstruction of the bile ducts. Its sensitivity is good for establishing the dilation of the bile ducts and the level of obstruction, but it is rarely helpful to determine the nature of the obstacle apart from a lithiasis pathology.

2.2.2.2.3 Thoraco-abdominal Computerized Tomography scanner (CT-scan)

The frequent infiltrating nature of cholangiocarcinoma and the absence of detectable mass limit the diagnostic performance of the CT scan. On the other hand, the performance of the CT scan is excellent for the visualization of lymphadenopathy and distant metastasis and for the evaluation of vascular staging.

2.2.2.2.4 Magnetic resonance cholangiopancreatography (MRCP)

The examination combines injected hepatic MRI sequences and cholangio-MRI sequences. It is the best performing imaging procedure for characterizing proximal biliary stenosis. In this situation, it makes it possible to have a biliary mapping which is essential before a possible ERCP or surgery. MRCP can raise the suspicion of stenosis in primary sclerosing cholangitis (PSC) or in IgG4-related sclerosing cholangitis which requires specific management. The performance for vascular extension and for metastatic assessment is similar to that of the CT scan. In a prospective randomized study the sensitivity and specificity for the diagnosis of malignant biliary stenosis were respectively 77% and 63% for the CT-scan versus 85% and 71% for the MRCP [11].

2.2.2.2.5 18F-flouro-deoxy-glucose PET (18F-FDG-PET).

Positron emission tomography (PET) and CT are diagnostic modalities combined in a PET/CT examination using 18F-FDG. The 18F-FDG-PET provides information about cellular metabolism, and CT visualizes morphological details of organs. The18F-FDG is a marker of glucose metabolism and areas of hypermetabolism are suspect for malignancy, especially if correlated with CT changes [12]

In a prospective study, involving 123 patients, 18F-FDG-PET did not show superiority over CT scan and MRI (MRI / MRCP) for the diagnosis of malignant biliary stenosis [13].

2.2.2.3 Endoscopic Ultrasonography (EUS) +/- Fine-needle Aspiration (FNA)

EUS most often can visualize stenosis of the extrahepatic bile ducts. But its performance is inferior for the visualization of stenosis of the supra-pancreatic part of the common bile duct and of the superior biliary convergence compared to stenosis of the distal common bile duct[14]. An irregular appearance or focal thickening of the bile duct wall of more than 3mm is associated with a diagnosis of cancer in indeterminate biliary stenosis [15]. However, these aspects do not definitively establish the presence or absence of cancer. Diffuse thickening of the wall of the main bile duct over a long segment suggests a non-neoplastic etiology, it can be observed in primary sclerosing cholangitis, IgG4-related sclerosing cholangitis but nevertheless also in cholangiocarcinoma [14]. The performance of EUS FNA was reported in a systematic review with meta-analysis including 20 studies and 957 patients. The sensitivity and specificity for the diagnosis of malignancy of a malignant stenosis were 80% and 97% respectively [16]. The benefit of EUS +/- FNA after no diagnostic ERCP with cytologic brushing for the diagnosis of malignancy in patients with biliary stenosis was reported in a systematic review with meta-analysis [17]. Ten studies, 6 prospective and 4 retrospectives, including 1162 patients fulfilling the inclusion criteria were retained as relevant. The incremental benefit of EUS +/- FNA over ERCP with cytologic brushing was 14%. EUS +/- FNA was helpful in diagnosis in one in seven patients with indeterminate biliary stenosis. The diagnostic impact appeared even greater for patients with distal stenosis or those related to nodules responsible for extrinsic compression. The mean diagnostic performance of cytology in these studies at the initial ERCP was 45%.

The risk of performing EUS-FNA for the diagnosis of bile duct stenosis could arise from the seeding of cancer cells along the needle path. This risk is low for tumors of the pancreatic head because the transduodenal path of the EUS-FNA needle will be completely resected during the pancreaticoduodenectomy. However, this risk must be considered for the EUS-FNA attempt of supra-pancreatic biliary stenosis. In this case, the EUS puncture needle passes through the peritoneum and the intraperitoneal fat which will not be resected surgically. The actual incidence of needle inoculation after EUS-FNA is difficult to determine. However, data are available for perihilar cholangiocarcinoma. In a study including patients for liver transplantation in whom staging laparotomy was performed, peritoneal tumor implants were found in 83% (5/6) of patients in whom EUS FNA or diagnostic biliary biopsy transperitoneal biopsy had been performed, compared to 8% (14/175) of patients without prior transperitoneal biopsy ($p < 0.01$) [18]. Although this was a retrospective study, these results suggest that tumor seeding may be a common event after EUS FNA for the diagnosis of perihilar biliary stenosis.

2.2.3 Diagnostic ERCP for characterization of indeterminate biliary stenosis

Cross-sectional imaging techniques and blood tests are rarely sufficient to make the diagnosis of biliary stenosis. In the absence of extrinsic compression by a visible nodule for which specialized diagnostic endoscopic techniques such as EUS-FNA will play a major role, the precise characterization of biliary stenosis can be difficult and require the use of a diagnostic ERCP.

Despite the risk of complications inherent in the technique, performing a diagnostic ERCP with the use of ancillary techniques available in 2021 is justified in order to reduce the rate of indeterminate biliary stenosis and to identify a non-neoplastic biliary stenosis potentially curable by medical or endoscopic treatment. The objective is to avoid the performance of potentially very morbid major surgery.

2.2.3.1 Direct biliary intraductal samples.

2.2.3.1.1 Endobiliary cytology by brushing

ERCP brush cytology was first described in 1975. It is currently the routine technique used in symptomatic biliary stenosis to make a diagnosis on a cytopathological basis. It is a safe and relatively simple diagnostic technique for an operator trained in ERCP [19 20]. However the diagnostic performance of the technique reported in three meta-analyses published between 2013 and 2015 remains disappointing due to a low sensitivity of between 41% and 45% [21 22 23]. Several technics to optimize the diagnostic performance were developed.

2.2.3.1.2 Modification of the brush design

In order to increase the diagnostic performance of endobiliary brush cytology, new generations of cytology brushes have been developed, but without a significant increase in performance [24] or with results reported in studies including a small number of patients [25].

2.2.3.1.3 FISH

Fluorescence in situ hybridization (FISH) can be used to detect chromosomal aneuploidy in biliary brushing samples and, could improve the sensitivity of cytology. However, it was suggested that its specificity was lower than conventional cytology: 89% vs 100% in a prospective study in 81 patients with bilio-pancreatic stenosis [26].

*2.2.3.1.4 **Combination of bile exfoliative and endobiliary brushing cytology: introduction to publications 1 and 2***

The specificity of biliary cytology is remarkably high for the diagnosis of malignant stenosis: 90% to 100%[27]. However his sensitivity is limited : 6 à 50 % [28]. The combination of endobiliary brushing and bile aspiration has been suggested to improve diagnostic performance in biliary stenosis [29]. We report our experience of the increasing of diagnosis accuracy in **publication 1** [30] and **publication 2** [31]

2.2.3.2 Direct endobiliary biopsy under fluoroscopic control

Histopathological examination is the gold standard for the diagnosis of tumors of the biliary ducts. Endobiliary direct biopsy under fluoroscopy with a biopsy forceps during the ERCP is

the procedure used in our second-line experience after cytological samples especially in the event of an endobiliary nodular tumor. Sensitivity of the direct endobiliary biopsy has been reported as significantly greater than that of the endobiliary brushing in a retrospective study of 241 patients: 61% vs 36% in particular for cholangiocarcinoma with a sensitivity of 79% vs. 42% [32]. However, in real life its sensitivity remains lower due to the inability to obtain adequate samples in case of infiltrating tumor. In a meta-analysis with 9 relevant studies including a total of 730 patients, the sensitivity of the per ERCP biopsy was 48% [23]
Proposals for modifying the shape of the biopsy forceps have been proposed, angled biopsy forceps[33] [34] with a sensitivity equal to 60% in the study that has evaluated[34]. Recently, the availability of a guide-wire biopsy forceps has allowed an easier introduction of the biopsy forceps at the level of the major papilla and a much secure progression in the bile ducts.[35]

2.2.3.3 Direct Combined methods

The combination of sampling techniques during the ERCP has been proposed to increase diagnostic sensitivity. The combination of cytological brushing and endobiliary biopsy has been reported in a prospective study conducted in 233 patients, noting that the biopsy could only be obtained in 55% of them with a sensitivity of 66% [36]
The repeated aspiration of bile by a naso-biliary drain left in place during the ERCP obtained a sensitivity of 72.4% after the completion of 6 samples [37]. The combination of a bile aspiration and an endobiliary biopsy made it possible to obtain a sensitivity of 77.9% [38]. The use of a basket in combination with endobiliary brushing would increase the sensitivity up to 86% [39]. However, all these techniques are more complicated and aggressive than a bile aspiration associated with cytological brushing, procedure that uses only one device during the ERCP.

2.2.3.4 Diagnostic devices through the working channel of the duodenoscope

2.2.3.4.1 Intraductal ultrasonography (IDUS)

Guidewire EUS high-frequency (20 MHz) mini probe is an imaging modality that showed promise for the assessment of biliary stenosis in the late 1990s.
Before its use during an ERCP, the IUDS was used by a transcutaneous access and compared to the practice of a per cutaneous cholangioscopic biopsy [40].
The accuracy of the technique was inferior to that of per cutaneous cholangioscopic biopsy, but its practice increased the sensitivity of the procedure for the diagnosis of malignancy.

In case of ampullary neoplasm, IDUS correctly assessed ductal infiltration of common bile duct and pancreatic duct in 90% of 33 patients included in a prospective controlled study [41]. Concerning the patients with a biliary stenosis without abdominal mass on CT scan, it has been suggested that IDUS performed during an ERCP increased accuracy for the diagnosis of malignancy to 90% compared to 58% for brushing cytology [42].

Moreover in a recent study performed in 65 patients [43], the use of the IDUS was proposed in an original way to intraductal biliary biopsy by ERCP by visualizing the thickest bile duct segments considered to be the areas most infiltrated by the tumor. The biopsy forceps were introduced into the main bile duct through the transpapillary tract under fluoroscopic control while keeping the IDUS mini probe in place. The accuracy was significantly higher with the intraductal biliary biopsy guided by IDUS than with the conventional intraductal biliary biopsy (90.8% vs 76.9%, p = 0.027). For the subgroup of 27 patients with an infiltrating biliary lesion, the IDUS-guided intraductal biliary biopsy had a significantly higher cancer detection rate than the conventional ERCP biopsy: 89.6% vs. 65.5%, (p = 0.028).

2.2.3.4.2 Probe-based confocal laser endomicroscopy (pCLE)

Cellvizio pCLE (Mauna Kea Technologies, Paris, France) is able to produce in real time microscopic images of bile duct wall generated by illuminating the examined tissue with an argon laser beam driven by micrometric optical fibers using a dedicated confocal mini-probe. An intravenous injection of fluorescein is required. The probe is introduced into the bile duct inside a carrier catheter and applied to the tissue to be examined under fluoroscopic or endoscopic control (through a choledocoscope). The resulting image resolution is 3.5 microns with a field of view of 325 microns. The depth of the tissue examined is 40 to 70 microns. The videos and images obtained live allow a histological evaluation in vivo in real time: "virtual biopsy". A consensual definition of the specific criteria for the results of biliary (and pancreatic) pCLE has been established and developed for indeterminate stenosis with the criteria allowing to differentiate a healthy bile duct from a malignant stenosis: the Miami classification [44]. The overall diagnostic accuracy of this classification was at 81% for the diagnosis of malignant stenosis, however, due to false positives, its low specificity at 67% limited its practical value [45]. The limited ability of the Miami Classification to differentiate malignant stenosis from inflammatory stenosis has been reported with significantly poorer accuracy in patients who had presented with cholangitis and / or in whom a stent was placed prior to pCLE by compared to the group without biliary procedure in the previous month, respectively 73% vs 87% [46] . Another classification taking these results into account has been proposed : the Paris classification [47]. A validation study of this classification has been published [48]. The rereading by 6 experts of 40 pCLE videos of biliary stenosis made it possible to obtain a better specificity at 83% without loss of sensitivity: 81% but with only passable inter-observer agreement (fair): kappa = 0.37.

2.2.3.4.3 **Cholangioscopic guided samples: introduction to publication 3**

For more than 15 years, peroral cholangioscopy has been shown to improve diagnostic accuracy for indeterminate biliary stenosis after a first inconclusive diagnostic ERCP including for the diagnosis of cholangiocarcinoma in primary sclerosing cholangitis with dominant stenosis. In the study by Fukuda et al, the sensitivity for cancer diagnosis compared to ERCP brushing cytology increased from 58% to 100% and the diagnostic accuracy from 78% to 93% in a series of 38 cholangiocarcinoma and 38 benign lesions [49]. The first studies were performed with “mother and baby” systems using a reusable cholangioscope. This technique has not been widely used because of the fragility of the device with high repair costs, the high cost of the investment for a limited number of uses, and the need to perform the procedure with two operators. The introduction of a single-use, single-operator (SOC) cholangioscope named Spyglass ® (Boston Scientific) in 2007 made cholangioscopy a much more accessible procedure for endoscopy platforms. Since the initial report by Chen et al showing the feasibility of this procedure in 35 patients [50], a large number of studies evaluating the diagnostic performance of SOC for the diagnosis of indeterminate biliary stenosis have been published. Two systematic reviews and meta-analyzes were published in 2015 [51] [52] with different inclusion criteria: the review by Navaneethan et al [51] included 10 retrospective and prospective studies including 456 patients, aiming to determine the value of cholangioscopic guided biopsies with the dedicated forceps Spybite ® , while that of Sun et al [52] which included 8 studies (335 patients), aimed to differentiate the value of visual impression from that of biopsies guided by cholangioscopy. For the diagnosis of malignant stenosis, the pooled sensitivity was 60.1% [51] and 69% [52], while the specificity of the biopsy with the dedicated biopsy forceps (Spybite ®) was 98% in both studies. However, Sun et al [52] determined that visual impression had a higher sensitivity for malignancy at 90% with specificity at 87%. Only four of the studies on which the two meta-analyzes were based included patients with indeterminate biliary stenosis: inconclusive ERCP and brushing [53] [54] [55] [56]. In the remaining studies biliary stenosis was difficult to diagnose after inconclusive CT and MRI, but results from ERCP with cytologic brushing were not available. The sensitivity and specificity of SOC in these four studies was respectively 74.7% and 93.3% (95% CI 85.1% to 97, 8%). We reported the impact of SOC in management of patients with indeterminate biliary stenosis in **publication 3** [57]

2.3 Therapeutic ERCP for benign biliary stenosis.

For nearly 3 decades, endoscopic treatment using ERCP is used for the treatment of biliary benign stenosis with first-line biliary drainage and etiological treatment. Surgery is considered only in case of failure of endoscopic and radiologic treatment for benign biliary stenosis. Interventional radiological treatment may be the first line treatment in case benign biliary stenosis in a patient with previous partial or total gastrectomy and/or presenting an intrahepatic stenosis on a small caliber biliary tract. In the other cases, ERCP is the first-line therapeutic method for performing stenosis calibration based on the placement of stents.

2.3.1 Benign stenosis without indication for therapeutic ERCP

Benign stenosis constituting exceptions to endoscopic treatment by ERCP are stenosis, due to autoimmune pancreatitis type 1 and 2, IgG4-related sclerosing cholangitis, rare vasculitis, sarcoidosis, histiocytosis. Theses stenosis will require non-interventional medical treatment.

2.3.2 Biliary stenosis of primary sclerosing cholangitis (PSC)

PSC is a special case in which ERCP has a prominent place in the treatment by performing dilations without placing a biliary stent. A dominant stenosis for European Society of Gastrointestinal Endoscopy (ESGE) and European Association for the Study of the Liver (EASL) should be defined as a stenosis with a diameter of ≤ 1.5 mm in the common bile duct and / or ≤ 1.0 mm in the hepatic duct within 2 cm of the main hepatic confluence [58]. Endoscopic balloon dilation of dominant stenosis has been shown to be effective with increased survival in patients [59]. The absence of a stent allows close endoscopic monitoring of stenosis by cholangioscopy in these patients at high risk of degeneration into cholangiocarcinoma. In the event of long stenosis or multiple early recurrences after dilation, short-term calibration by placing a plastic stent may be indicated for a period of one to two weeks [60].

2.3.3 Benign biliary stenosis: treatment with ERCP + stent

2.3.3.1 Postoperative including post liver transplantation stenosis

These are mainly post cholecystectomy stenosis and post liver transplant stenosis. The incidence of post-cholecystectomy stenosis is 0.15% –0.36% with a decreasing trend over the past two decades [61 62], they occur after very variable delays (from one day to several years), generally following the installation of a clip or an electrocoagulation close to the bile duct. Biliary complications are the most frequent complications after liver transplantation, with an incidence of 10 to 25% [63]. These complications are observed in liver transplant both from deceased donor and from living donor representing an important cause of morbidity and mortality in liver transplant recipients. Up to 80% of biliary stenosis develop at the anastomotic site [64 65]. Different risk factors have been identified such as the duration of cold ischemia, history of postoperative bile leakage, the diameter of the main bile ducts and the age of the recipient [66].

2.3.3.2 Chronic pancreatitis

The occurrence of biliary stenosis during the evolution of chronic pancreatitis is reported in 3 to 23% of patients, with a prevalence of up to 46% [67]. Symptoms may include jaundice, lithiasis of the common bile duct and cholangitis. Jaundice resolves spontaneously in 20 to 50% of patients within several week, due to disappear of the edema of the head of the pancreas in acute flare-ups, but the occurrence of secondary biliary cirrhosis was reported : 7, 3% of 288 patients in a review of 11 studies [67].

2.3.3.3 Alveolar echinococcosis: introduction to publication 5

This is a much rarer cause of biliary stenosis. Human alveolar echinococcosis is a rare parasitic disease caused by the development of the larval form (metacestode) of the cestode Echinococcus multilocularis. Endemic areas are located in Central and Eastern Europe, North America, Central Asia, China, Japan [68]. In Europe, in highly endemic areas, the annual incidence of alveolar echinococcosis is 2/100 000 [69]. The larva of the parasite slowly progresses through the liver like a tumor, causing a granulomatous reaction and intense fibrosis. The biliary tree is often affected by the parasitic process and / or compressed by fibrosis. The aggressive course of this parasitic infection should give rise

to talk of non-neoplastic stenosis and not of benign stenosis, the course at the loco-regional level and at a distance can be as severe as a cancerous pathology.
In advanced cases of alveolar echinococcosis with complications related to bacterial superinfection or communication of the necrotic cavity with the bile ducts, surgical resection is the only effective treatment [70]. Liver transplantation is an alternative to locally advanced stages when curative hepatectomy is not possible [71]. In 70% of cases, only palliative treatment can be offered combining parasitostatic treatment with albendazole and treatment of complications of the disease. Currently the expert consensus is in favor of surgical abstention whenever the lesions cannot be completely resected [70]. Endoscopic drainage by ERCP is an alternative to percutaneous biliary drainage (PTBD) with the advantage of avoiding the discomfort of an external bile drain [72]. Our experience with ERCP treatment of biliary stenosis related to alveolar echinococcosis is reported in **publication 5** [73]

2.3.4 Treatment of benign biliary stenosis by ERCP and placement of cSEMS: introduction to publication 4

Up to 1990, the endoscopic treatment of postoperative stenosis in particular post cholecystectomy, was based on the dilation and placement of plastic (polyethylene) stents changed regularly every 3 -4 months by increasing the number of stents as much as possible for an average of 1 year [74] [75] with a two-year stenosis recurrence rate of 20% [75]. Given the risks and costs of this treatment linked to the high number of endoscopic procedures, the use of cSEMS for the treatment of benign biliary stenosis was considered. To evaluate the safety of this procedure, a study was performed in 10 mini pig in 1991 [76]. Fully covered SEMS were endoscopically placed, let in place 3 months and removed endoscopically. Removability was easy and no significant histologic lesion was noticed on the common bile duct. In human both partially and fully covered metallic stents were used, mostly fully covered. These cSEMS are easier to put in place and remain permeable longer than plastic prostheses. The use of cSEMS for the treatment of benign postoperative biliary stenosis and secondary to chronic pancreatitis reduces the number of procedures. Our experience with ERCP treatment of biliary stenosis with cSEMS is reported in **publication 4** [77].

3 Publication 1 [30]

Monocentric study of bile aspiration associated with biliary brushing performed during Endoscopic Retrograde Cholangiopancreatography in 239 patients with symptomatic biliary stricture

Michèle Fior-Gozlan; Diane Giovannini; Maud Rabeyrin; Anne Mc Leer-Florin; Marie-Hélène Laverrière; **Philippe Bichard**

Cancer Cytopathol 124, 330–339 (2016).

For the diagnostic of biliary stenosis, sensitivity of biliary cytology during ERCP was considered as highly specific but with a low sensitivity. This study evaluated the diagnostic performance of ERCP with biliary stenosis brushing combined with bile aspiration before and after brushing. The study was performed retrospectively from a prospective data base of a tertiary center from 2004 to 2009 in 239 consecutive patients. The definitive diagnostic of malignancy was obtained by biopsy, surgery, fine-needle aspiration or disease course. A pancreatic adenocarcinoma was present in 50% and a cholangiocarcinoma in 23%

Results

- Sensitivity of bile aspiration alone was 56.4% and specificity was 93.9%
- Sensitivity of brushing alone was 62.5% and specificity was 100%
- **For bile aspiration and brushing combined**, sensitivity was 81% and specificity was 100%

Impact for ERCP practice

This study suggested than in patients with biliary stenosis, the combination of bile aspiration and biliary brushing during ERCP was a simple, and safe procedure that increased the diagnosis of malignant biliary stenosis, with an 81% sensitivity. One limitation of the study was the high percentage of pancreatic adenocarcinoma. Indeed, for distal common bile duct stenosis due to a nodule detected by CT scan or MRI, the performance of a transduodenal EUS-FNA has an accuracy better than 90% [78]. Nevertheless, this procedure appeared of a great interest for the diagnosis of cholangiocarcinoma in which the diagnostic efficiency of EUS-FNA is poor.

Original Article

Monocentric Study of Bile Aspiration Associated With Biliary Brushing Performed During Endoscopic Retrograde Cholangiopancreatography in 239 Patients With Symptomatic Biliary Stricture

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BACKGROUND: The cytologic diagnosis obtained by brushing or biopsy in malignant biliary strictures is considered to be highly specific but poorly sensitive. The diagnostic association of biliary brushing and bile exfoliate cytology has been suggested but is rarely performed in clinical practice. The objective of this study was to assess the diagnostic performance of bile aspiration associated with biliary brushing during therapeutic endoscopic retrograde cholangiopancreatography (ERCP). **METHODS:** From 2004 to 2009, 239 consecutive patients who underwent ERCP were included in the study. The biliary strictures were considered clinically benign in 26% of patients, uncertain in 25%, and malignant in 49%. The 298 cytologic samples collected were divided in 3 groups: bile aspiration alone (26%), biliary brushing alone (20%), and bile aspiration combined with brushing (54%). The definitive diagnosis of malignancy was obtained by biopsy, surgery, and fine-needle aspiration or was determined by an unfavorable disease course. **RESULTS:** The cytologic diagnoses were as follows: 149 samples were benign (50%), 114 were malignant (38%), 34 had atypia (12%), and 1 had no diagnostic value. The procedure output values were as follows: for bile aspiration alone, sensitivity was 56.4%, specificity was 93.9%, the positive predictive value (PPV) was 91.7%, and the negative predictive value (NPV) was 64.6%; for brushing alone, sensitivity was 62.5%, both specificity and the PPV were 100%, and the NPV was 73%; and, for bile aspiration and brushing combined, sensitivity was 81%, both specificity and the PPV were 100%, and the NPV was 75%. **CONCLUSIONS:** For patients who have symptomatic biliary stricture, bile aspiration during ERCP is a simple and safe procedure. Bile aspiration combined with brushing significantly increases the yield of cytology for malignant biliary tumors (sensitivity, 81%), particularly in cholangiocarcinomas. ***Cancer Cytopathol* 2016;124:330-9**. *© 2015 American Cancer Society.*

KEY WORDS: bile aspiration; biliary stricture; brushing; cholangiocarcinoma; cytology; endoscopic retrograde cholangiopancreatography (ERCP).

INTRODUCTION

The incidence of cholangiocarcinoma and pancreatic adenocarcinoma has increased in the last 30 years, and the prognosis for patients with these tumors remains dramatically poor.[1] These carcinomas usually present with an extrahepatic bile duct (common hepatic duct and common bile duct) stenosis, mainly revealed by painless jaundice.

Abdominal ultrasonography, computed tomography, biliopancreatic endoscopic ultrasound (EUS), and magnetic resonance cholangiopancreatography are noninvasive imaging techniques that usually are performed to

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We thank Marylène Lorinet, Cécile Gozlan, and Eric Kendjo.

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Received: August 10, 2015; **Revised:** November 16, 2015; **Accepted:** November 17, 2014

Published online December 23, 2015 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cncy.21667, wileyonlinelibrary.com

evaluate the extent of a bile duct stenosis[2]; but, endoscopic retrograde cholangiopancreatography (ERCP) enables a cytologic or histologic diagnosis and can also be used to treat stenosis using stents.[3] However, sampling troubles frequently occur because of a proximally located stenosis, insufficient cellularity of a sample, or poor sample quality. Most often, stenoses are malignant, mainly because of pancreatic ductal adenocarcinoma or cholangiocarcinoma.[4] The etiology of benign stenosis includes lithiasis, acute or chronic pancreatitis, postoperative biliary strictures, or primary sclerosing cholangitis; and the latter frequently presents as cytologic atypia mimicking malignancy. Treatment options vary from palliative stenting or radiotherapy/chemotherapy to curative resection or transplantation and are sometimes based only on cytologic diagnosis when tumors are extensive and unresectable.[3]

Brushing cytology during therapeutic ERCP is the most commonly used procedure reported in the literature for assessing the diagnosis of biliary strictures. The specificity of brush cytology is remarkably high (range, 90%-100%),[5] but its sensitivity for malignancy varies between different series (range, 8%-83%).[6,7] Likewise, bile exfoliative cytology has variable, low sensitivity (range, 6%-50%) but high specificity (range, 99.5%-100%).[8–10] Biliary duct biopsy sensitivity is also relatively low (range, 36%-43%).[11–13] Combining biliary brushing and bile exfoliate has been suggested as a way to improve the diagnostic yield. In particular, in their 1993 study of 100 patients, Kurzawinski et al demonstrated the increased sensitivity of combined bile and brush cytology compared with bile cytology alone.[8] However, this combination is rarely used in clinical practice.[8–10]

Therefore, the objective of the current study was to assess the diagnosis performance of bile aspiration in combination with biliary brushing during ERCP. The results reported here confirm that that bile aspiration during ERCP is a simple and safe procedure and that bile aspiration combined with biliary duct brushing significantly increases the yield of cytology for malignant biliary strictures, particularly cholangiocarcinoma.

MATERIALS AND METHODS

Patients

Between January 1, 2004 and December 31, 2009, 1105 bile aspiration specimens and/or biliary brushings were obtained under ERCP at the Grenoble University Hospital. Two hundred thirty-nine consecutive patients who had 4 years of clinical follow-up were included in this study; including 42 of 239 patients who had more than 1 cytology specimen (range, 2-5 cytology specimens; total, 298 cytology specimens). For 76 specimens, there was a suspicion of benign disease; for 147 specimens, the presentation was in favor of malignancy; and, for 75 specimens, the radiologic presentation did not provide any diagnosis.

Intervention and Technique

Patients underwent ERCP under general anesthesia on an x-ray table using a video side-viewing duodenoscope positioned opposite the second duodenum main duodenal papilla. Access to the biliary tract was obtained through a sphincterotome, allowing an injection of the contrast medium (Telebrix; Guebert; France) and the introduction of a 0.035-inch-diameter guide wire into the biliary tract. The opacification confirmed the existence of a biliary duct stenosis and guided the diagnosis. The guide wire was passed through the stenosis. Most patients underwent biliary sphincterotomy to facilitate access to the biliary tract. In patients who had very tight stenoses, dilatation of the stenosis was performed with a hydraulic balloon measuring 6 or 8 mm in greatest dimension. A disposable cytology brush (Fig. 1A) protected by an 8-French size catheter was slipped over the guide wire through the stenosis. Two devices were used: Rx Combocath (Boston Scientific, Marlborough, Mass) and the Fusion Cytology Brush (FS-CB-1.5; Cook Medical, Marseilles, France).

Cytologic samples were collected before stenosis opacification and dilatation. Ten movements back and forth in the stricture were performed. The brush was immediately smeared on 1 or 2 slides by a nurse and then fixed in a 50% ethanol/50% acetone solution. The brush was then shaken and immersed in PreservCyt solution (Hologic France, Villepinte, France). From 3 to 10 mL of bile were collected above the stenosis in a sterile dry tube by aspiration using 1 of the channels of the sphincterotome or the catheter of the cytology brush (Fig. 1B). The bile was specifically collected above the stenosis; whereas the bile below the stenosis was eliminated in the duodenum after sphincterotomy. Because the length of the guide-wire tract at the distal end of the device was 6 cm, the entrance hole of the guide wire had to be above the stenosis to optimize bile aspiration. From 2007 onward, the endoscopists

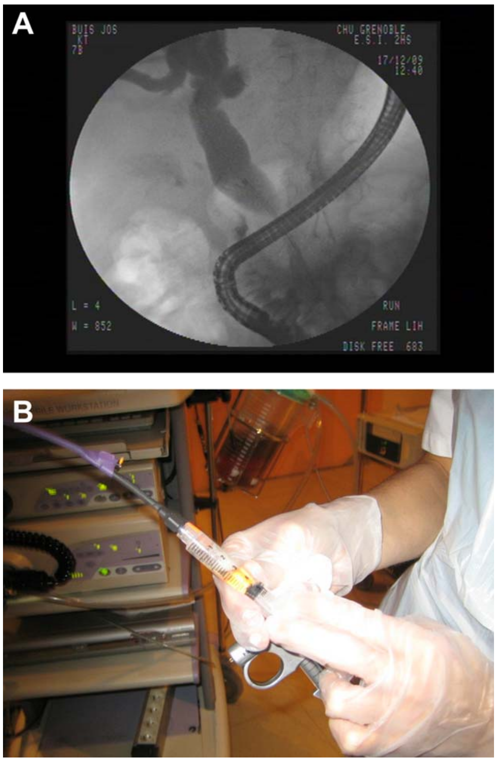

Figure 1. (A) A cytology brush slipped onto the guide wire is positioned above the stricture during endoscopic retrograde cholangiopancreatography (ERCP). (B) For bile aspiration, 3 to 10 mL of bile was collected before the stenosis in a sterile dry tube during ERCP.

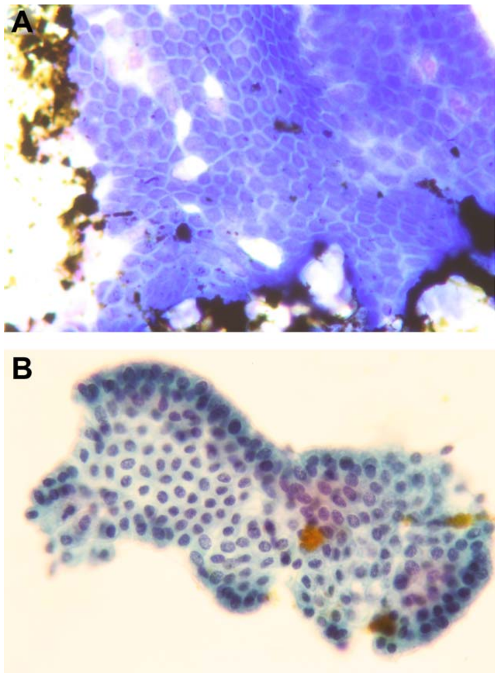

Figure 2. (A) A sample of benign biliary duct epithelium has monolayered sheets of cubical cells with regular nuclei in a honeycomb arrangement (bile; May-Grunewald-Giemsa stain, original magnification ×40). (B) Another sample of benign biliary duct epithelium has palisading nuclei with inconspicuous nucleoli and biliary pigments (bile; Papanicolaou stain, original magnification ×40).

collected 2 bile samples: 1 before and 1 after brushing.[14] The procedure was completed by biliary stenting.

Cytopathologic Examination

Bile aspiration specimens were cooled on ice and sent to the pathology laboratory as soon as possible to avoid degenerative changes.[15] Bile was centrifuged (at 2000 revolutions per minute for 10 minutes), and the deposit was smeared onto slides. Because Papanicolaou and May-Grunewald-Giemsa (MGG) stains are complementary for diagnosis, 2 slides were stained with Papanicolaou stain after using a Merckofix fixing spray (Merck France, Calais, France), and 2 slides were air-dried and stained with MGG stain. In addition, PreservCyt solution was used for ThinPrep slides (Hologic France) using Papanicolaou stain. The slides from brushing were stained with Papanicolaou stain.

Cytologic Interpretation

Samples were evaluated by an experienced cytopathologist and were interpreted according to the characteristics established by several investigators using standard cytologic criteria for malignancy.[16] The original diagnoses fell into 5 distinct groups: 1) "benign," 2) "with reactive atypia," 3) "with atypia suspicious for malignancy," 4) "malignant," and 5) "no diagnostic value." Only samples in group 4 were considered positive for malignancy.

Briefly, in the benign sample group (Fig. 2A,B), cohesive, homogeneous, small-to-medium epithelial cells were observed in a honeycomb arrangement; the cells had a round and regular nucleus with a low nucleocytoplasmic

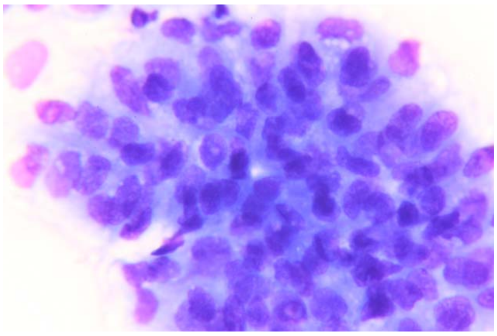

Figure 3. An atypical reaction of ductal epithelium reveals the cells with a variable nucleocytoplasmic ratio and finely granular chromatin with few nucleoli (bile; May-Grunewald-Giemsa stain, original magnification ×63).

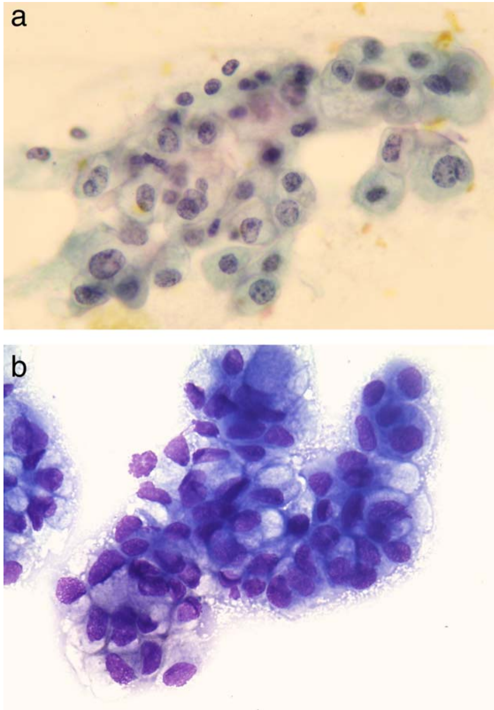

Figure 4. (A) This adenocarcinoma has pleomorphic nuclei, a high nucleocytoplasmic ratio, irregular nuclear outlines, prominent nucleoli, and coarse chromatin (bile; Papanicolaou stain, original magnification ×63). (B) Another adenocarcinoma has 3-dimensional clusters, loss of polarity of the nuclei, nuclear overlap, and intracytoplasmic vacuoles (bile; May-Grunewald-Giemsa stain, original magnification ×63).

ratio, a fine chromatin structure, no individualized nucleolus, and an indistinguishable membrane.[5,11,17] In the group "with reactive atypia" (Fig. 3), the ductal epithelial cells were discreetly heterogeneous with a slight anisonucleosis, and nuclei had a discreetly discernible membrane and sometimes a small nucleolus. However, nuclei remained polarized, and the chromatin was always finely distributed. The presence of neutrophils within the ductal epithelium also was correlated with benignity.[18] In the "suspicious group," either only a few cytologic criteria of malignancy were noted or only a few atypical cells were observed. In the "malignant group," adenocarcinoma was the usual diagnosis (pancreatic adenocarcinoma extending to the bile duct and cholangiocarcinoma). It is not actually possible to differentiate these 2 carcinomas on cytologic samples, because they have the same nuclear characteristics. The criteria used for malignant diagnoses were based on nuclear characteristics: 1) irregularly clumped chromatin, 2) disruption of nuclei with loss of polarity and nuclear overlap, 3) thickening and irregularity of the nuclear membrane (multiple authors emphasize this characteristic), 4) increased nucleocytoplasmic ratio, and 5) the existence of 1 or more nucleoli. The cells were cubic, isolated, or formed acini, cords, or 3-dimensional clusters (Fig. 4A,B). Well differentiated, moderately differentiated, and poorly differentiated adenocarcinomas were distinguished. For rare squamous carcinomas, endocrine carcinomas, metastases, and lymphomas, immunocytochemistry on stained slides was performed to confirm the diagnosis. In the group of samples with "no diagnostic value," specimens were acellular or with fixative artifacts, such as nuclear swelling (increased volume) and/or fragmented chromatin.

Statistical Analysis

All collected data were recorded in an electronic database. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy of each technique were calculated using the Stata version 12 software package (Stata Corporation, College Station, Tex).

RESULTS

Among the 239 patients who underwent ERCP for the diagnosis and treatment of a symptomatic biliary stricture in our institution, 143 were men (mean age, 67 years;

range, 25-98 years), and 96 were women (mean age, 68 years; range, 18-94 years). Forty-two of 239 patients had more than 1 cytology specimen (range, 2-5 cytology specimens), and there was a total of 298 specimens.

The cytologic diagnoses were performed on bile aspirations alone (79 cytologic samples; 26% of samples), or on biliary brushings alone (59 samples; 20% of samples), or on bile aspirations combined with brushings (160 samples; 54% of samples). The cytologic diagnoses were as follows: 149 samples were benign (50%), 114 were malignant (38%), 34 had atypia (12%; 22 classified as reactive atypia, and 12 classified as suspicious atypia), and 1 had no diagnostic value (acellular brushing). In this study, 45% of samples that were initially classified as reactive atypia and 25% of those initially classified as suspicious atypia were identified as benign at the final diagnosis. However, 55% of the samples that initially were classified as reactive atypia and 75% of those initially classified as suspicious atypia were revealed as malignant in a subsequent control assessment.

Initially, only specimens with benign and malignant cytologic diagnoses were retained for the statistical analysis: these included 263 specimens from 218 patients, because some patients underwent several cytologic samplings. Thus, for the current assessment, 72 patients had only bile specimens, 48 patients had only brushing specimens, and 143 patients had both bile and brushing specimens (Fig. 5.).

The final diagnoses for the 218 patients were assessed by histology (26 cephalic duodenopancreatectomies, 32 intraductal and papilla biopsies, 2 liver biopsies, and 19 metastasis biopsies), fine-needle aspiration (FNA) (16 pancreatic FNAs under EUS and 11 metastases FNAs), or long-term clinical follow-up (up to 4 years) for 112 patients. In 142 patients (65%), a diagnosis of malignancy was retained, including mainly pancreatic adenocarcinoma (n = 72), cholangiocarcinoma (n = 33), and metastasis (n = 26). For 76 patients (35%), a benign diagnosis was retained, including mainly lithiasis (n = 19), acute and chronic pancreatitis (n = 19), and primary sclerosing cholangitis (n = 7) (Table 1).

Of the 72 bile-only samples that had a definitive diagnosis available, 24 had a malignant cytologic diagnosis, and 48 were classified as benign. Of 48 brushing-only samples with a definitive diagnosis available, 15 were diagnosed as cytologically malignant, and 33 were classified as benign. Of 143 combined samples of bile and brushings, comprising the group on which the current study was focused, 75 had a malignant cytologic diagnosis, and 68 were classified as benign. No false-positive results were reported in this last group or in the group with brushings alone. Two false-positive results were obtained in 2 bile aspiration samples in relation to lithiasis. True-positive and false-positive results and true-negative and false-negative results for bile aspirations alone, brushings alone, and combined bile aspirations and brushings are summarized in Table 2. The sensitivity for accurate diagnosis with bile alone it was 56.4%, the specificity was 93.9%, the PPV was 91.7%, and the NPV was 64.6%; and the overall accuracy of bile alone was 73.6%. The sensitivity for accurate diagnosis with brushing alone was 62.5%, the specificity and the PPV were both 100%, the NPV was 73%; and the overall accuracy of brushing alone was 81.3%. The sensitivity for accurate diagnosis with combined cytologic analysis of bile and brushing was 81%, the specificity and the PPV were both 100%, the NPV was 75%; and the overall accuracy of bile and brushing combined was 87.4%. These results are detailed in Table 3.

In a second analysis, we included samples with reactive atypia (n = 22) that had benign diagnoses and samples that were classified as suspicious atypia (n = 12) that had malignant diagnoses. The sample with no diagnosis value (acellular brushing) was not taken into account. This left 297 samples (ie, 79 bile only, 58 brushing only, and 160 bile and brushing combined) (Fig. 5). These 297 samples corresponded to 239 patients (a control assessment was performed for the patient who had an acellular brushing, which revealed malignancy). The final diagnoses for the 239 patients were assessed as described above. In 156 patients, a diagnosis of malignancy was retained. In 83 patients, a benign diagnosis was retained (Table 1). True-positive and false-positive results and true-negative and false-negative results from bile aspiration alone, brushing alone, and combined bile aspiration and brushing are summarized in Table 2. The different sensitivities, specificities, PPV, PPV, and accuracy are detailed in Table 3.

Safety

No serious complications related to bile aspiration or brushing samples were recorded during the entire follow-up.

DISCUSSION

Treatment options for patients with malignant biliary stricture vary from palliative stenting or radiotherapy/chemotherapy to curative resection or transplantation and

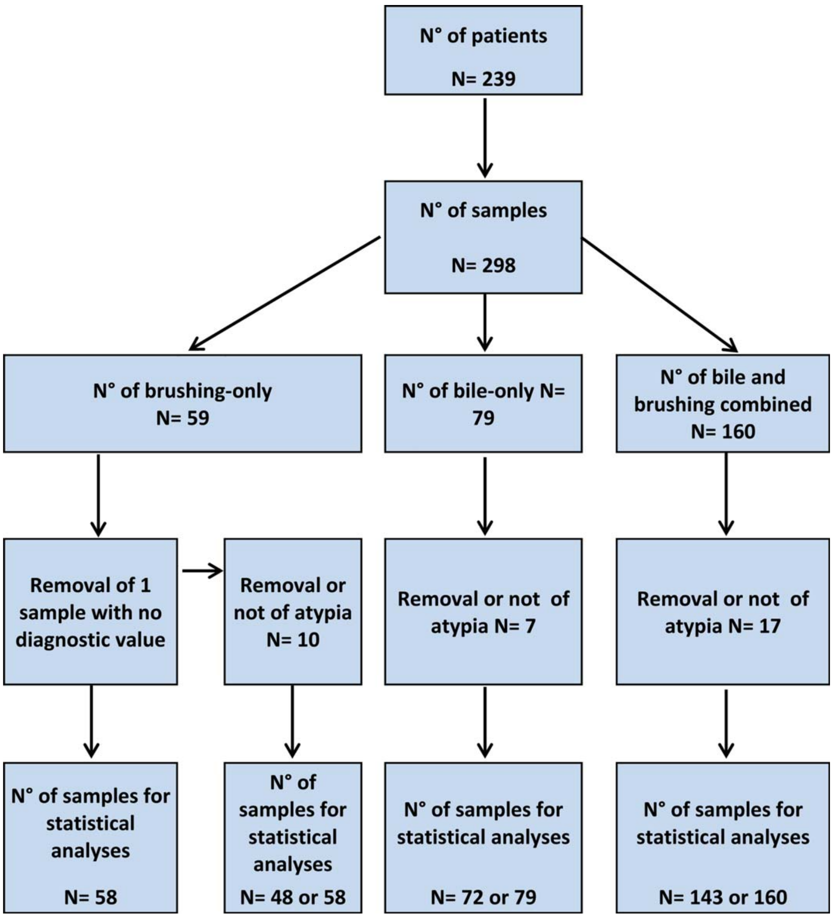

Figure 5. This is a flow chart illustrating the samples assessed in the current study for the diagnostic performance of bile aspiration associated with biliary brushing during therapeutic endoscopic retrograde cholangiopancreatography. The cytologic samples collected were divided in 3 groups: bile aspiration alone, biliary brushing alone, and bile aspiration combined with brushing.

are sometimes based only on a cytologic diagnosis when the tumor is extensive and unresectable.[3] Brush cytology during ERCP is the most commonly used procedure described in the literature for assessing the diagnosis of biliary strictures. Its specificity is remarkably high, ranging from 90% to 100%,[5] but its sensitivity for malignancy varies according to series, ranging from 8% to 83%.[6,7]

The diagnostic association of biliary brushing cytology and bile exfoliate cytology has been suggested, but the combination is rarely used in clinical practice. Only a few studies on the diagnostic value of this association are available. Our current single-center study, based on a comparative analysis of 298 specimens performed on 239 patients over 6 years, is 1 of largest series analyzing bile aspiration combined with biliary brushing during ERCP.

According to the literature, despite their low sensitivity, biliary duct biopsies are considered the gold standard.[11–13] However, in view of our results, it may be more appropriate to consider the cytologic combination of biliary brushing and bile as the gold standard instead. Indeed, herein, we have demonstrated that the sensitivity of this combination is high, reaching 81%, and is superior to the sensitivity of 69% reported previously by Kurzawinski et al.[8]

The high sensitivity we obtained can be explained, among other things, by the close cooperation between the cytopathologist and the gastroenterologist[19] to avoid any sampling protocol mistakes and to ensure optimal clinical information,[20] because the latter is critical to obtaining a correct interpretation of cytologic samples. For example, it is necessary to know whether the patient has had any prosthesis changes, because prostheses can provoke reactive atypia, which is sometimes difficult for the cytologist to interpret and can be responsible for false-positive results. It is also important to know whether the patient has been followed for any another malignant pathology possibly involving pancreatic metastases.

Another explanation for the high sensitivity obtained in our study is that fixation artifacts on brushing slides smeared by the endoscopists can be compensated by the results from bile aspiration slides prepared by the laboratory cytotechnicians. However, using the ThinPrep technique can greatly improve the quality of slides that are smeared by the endoscopists.[18,21,22]

The relatively low number of false-negative results may be attributed to our use of 2 complementary stains for the diagnosis: Papanicolaou and MGG. The combination of bile aspiration and brushing, as we emphasized in 2006,[23] increases the diagnostic performance, and the bile cellularity is very important (Fig. 6). Endoscopists from our institution have been performing bile aspiration before and after biliary brushing since 2007 and have demonstrated that the sensitivity of bile cytology before and after brushing is not significantly different (75.3% vs 68.8%, respectively).[14]

Our expertise in this kind of biliary cytology since 2004 urges us to recommend the combination of bile and

TABLE 1. Final Benign and Malignant Diagnoses in 218 Patients Who Had Samples Without Atypia and 239 Patients Who Had Samples That Included Atypia

Diagnosis	No. of Patients (%) Without Atypia	Including Atypia
Malignant	142 (65.2)	156 (65.3)
Pancreatic adenocarcinomas	72	76
Cholangiocarcinomas	33	37
Pancreatic metastases	26	31
Adenocarcinoma of the papilla	8	8
Malignant clinical course or death	2	2
Intraductal papillary-mucinous carcinoma	1	1
Neuroendocrine carcinoma		1
Benign	76 (34.8)	83 (34.7)
Cholelithiasis	19	20
Chronic pancreatitis	18	20
Benign clinical course	15	18
Postoperative biliary strictures	13	13
Primary sclerosing cholangitis	7	8
Acute pancreatitis	1	1
Hepatorenal polycystic	1	1
Ischemic cholangitis	1	1
Septic shock	1	1

TABLE 2. True-Positive, False-Positive, True-Negative, and False-Negative Results for the 3 Groups of Samples

Sample Type	No. of Samples	No. of Results TP	FP	TN	FN
Bile aspiration					
Without atypia	72	22	2	31	17
Including atypia	79	25	3	32	19
Brushing alone					
Without atypia	48	15	0	24	9
Including atypia	58	17	2	26	13
Bile and brushing					
Without atypia	143	75	0	50	18
Including atypia	160	79	0	57	24

FN indicates false-negative; FP, false-positive; TN, true-negative; TP, true-positive.

TABLE 3. The Different Sensitivities, Specificities, Positive Predictive Values, Negative Predictive Values, and Accuracy With 95% Confidence Intervals for the 3 Groups of Samples

Sample Type	No. of Samples	Rate (95% CI), % Sensitivity	Specificity	PPV	NPV	Accuracy, %
Bile aspiration						
Without atypia	72	56.4 (40.8-72)	93.9 (85.8-100)	91.7 (82.6-100)	64.6 (51.1-78.1)	73.6
Including atypia	79	56.8 (42.2-71.5)	91.4 (82.2-100)	89.3 (77.8-100)	62.7 (49.5-76)	72.2
Brushing alone						
Without atypia	48	62.5 (43-82)	100	100	73 (58-88)	81.3
Including atypia	58	57 (39-74)	93 (83-100)	89 (76-100)	67 (52-81)	74.4
Bile + brushing						
Without atypia	143	81 (73-89)	100	100	75 (63-84)	87.4
Including atypia	160	77 (69-85)	100	100	70 (60-80)	85

CI indicates confidence interval; NPV, negative predictive value; PPV, positive predictive value.

brushing as more reliable than brushing alone. The cellularity of the bile with the bile-and-brushing combination is greater than that with brushing alone. Indeed, brushing the stenosis causes biliary cells to shed into the bile, which is then aspirated, increasing the bile cellularity and thus improving the diagnosis. However, a current study in our hospital in collaboration with endoscopists highlights the importance of bile cellularity compared with that from brushings in the bile-and-brushing combination (Fig. 6).

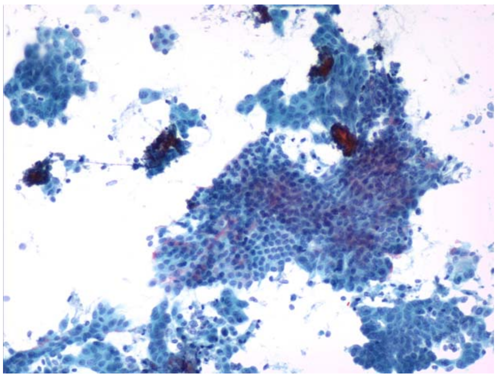

Figure 6. This representative sample reveals the high cellularity of the bile. Two cell populations are present: the benign biliary epithelium and the adenocarcinoma at the periphery (Papanicolaou stain, original magnification ×10).

If we incorporate the samples that were classified as reactive atypia into the group of benign samples and incorporate the samples that were classified as suspicious atypia into the group of malignant samples, then, with regard to combined bile and brushing, the specificity is maintained at 100%, but the sensitivity is decreased (from 81% to 77%) along with the NPV (from 75% to 70%), which is prejudicial for the patient. In our study, 45% of samples that were classified as reactive atypia and 25% of those classified as suspicious atypia were finally diagnosed as benign, in agreement with the literature reporting false-positive results in patients with *Fasciola hepatica* infection,[24] prolonged biliary stenting,[25,26] xanthogranulomatous cholangitis and related pseudotumor,[27] sarcoidosis,[28] and primary sclerosing cholangitis.[29] Two false-positive results were obtained in 2 bile aspiration samples that were related to lithiasis. Finally, 55% of samples that were classified as reactive atypia and 75% of those classified as suspicious atypia were diagnosed as malignant during a subsequent

TABLE 4. The Combination of Several Samples Improves Sensitivity: Summary of a Small Review of the Literature

Reference and Biliary Sampling Technique	No. of Patients	Sensitivity of Biliary Sampling Technique, %
Kurzawinski 1993[8]		
Bile alone	47	33
Brushing + bile	46	69
Ponchon 1995[12]		
Brushing	210	35
Intraductal biopsy	128	43
Brushing + intraductal biopsy	115	63
Farrell 2001[30]		
Brushing	24	57
Brushing + stricture dilatation + endoscopic needle aspiration	22	85
Dumonceau 2007[31]		
Brushing	30	52
Stricture dilatation and grasping basket	30	86
Uchida 2008[32]		
Bile aspiration through an ENBD tube	98	
First sample		40.8
After 6 repeated cytologic samplings		72.4
Asioli 2008[33]		
Histologic sectioning of brush bristles for 20–25 sections	112	91
Curico 2012[34]		
Brushing	42	37.5 (for all samples)
Intraductal aspiration		89
Kim 2014[35]	766	
Bile alone		24.7
Endobiliary forceps biopsy		74.4
Bile + endobiliary forceps biopsy		77.9

ENBD tube indicates endoscopic nasobiliary drainage tube.

control assessment. In light of these findings, a histologic or cytologic control assessment is performed in samples that have an atypical diagnosis.

The combined use of several sample types has been reported previously, and those results are summarized in Table 4. For instance, Ponchon et al obtained a sensitivity of 63% by combining brushing and intraductal biopsy.[12] Farrell et al combined brushing and endoscopic needle aspiration to obtain a sensitivity of 85%.[30] Uchida et al reported that sensitivity was 40.8% for bile aspiration using an endoscopic nasobiliary drainage tube, but sensitivity reached 72.4% after 6 repeated samplings.[32] More recently, Kim et al combined bile aspiration and endobiliary forceps biopsy to obtain a sensitivity of 77.9%.[35] Several other procedures are under development and are summarized in Table 4, such as grasping basket, with a sensitivity of 86%[31]; cell blocks of the brushings, with a sensitivity of 91%[33]; and intraductal aspiration, with a sensitivity of 89%,[34] which could increase the recovery of exfoliated malignant cells. However, all of these methods are more complicated and aggressive than a simple bile aspiration before and after brushing during a therapeutic ERCP.

Recently, the use of brush cytology specimens has been expanded to the study of molecular markers and DNA-based testing. Mutations in the genes *p53* and v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) are observed in biliary malignancy[36]; however, to our knowledge, brush cytology has not been used to date for mutation analyses. Efforts remain to be made to develop the testing of biliary cytology using image-based testing of DNA histograms for ploidy analysis. Combined digital imaging analysis and fluorescence in situ hybridization has been evaluated, and the results indicate that the sensitivity and specificity are intermediate between routine cytology and fluorescence in situ hybridization.[36,37] Translocations in the ROS proto-oncogene 1, receptor tyrosine kinase (*ROS1*) have been observed in cholangiocarcinoma[38] as well as B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) mutations,[39] and the French molecular genetics platforms are engaged in a research program to test for these alterations (the French National Cancer Institute Secure Access Program to Innovate Targeted Therapies [ACSe Program]).[40] The mutated tumors that have been identified were in intrahepatic cholangiocarcinoma.[38,39] Further studies are needed to evaluate the possibility of using cell blocks of brushing and bile samples for these molecular analyses.[41]

Conclusion

Optimal cytodiagnosis depends on multiple factors, including tumor location; type of neoplasm, endoscopist, and cytopathologist skills; and quality of the cytologic material.[11,42,43] The combination of several samples improves the diagnostic sensitivity for malignancy. In patients with symptomatic biliary stricture, the aspiration of bile before and after brushing of the stricture during therapeutic ERCP is a simple, cheap, and safe procedure that increases the cytologic diagnosis of malignant biliary strictures, with sensitivity increased to 81%. This is a procedure of a great interest for the diagnosis of malignant strictures, especially for cholangiocarcinomas, which are poorly investigated using classical techniques.

FUNDING SUPPORT

No specific funding was disclosed.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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4 Publication 2 [31]

Performance of bile aspiration plus brushing to diagnose malignant biliary strictures during endoscopic retrograde cholangiopancreatography.

Gael S Roth, **Philippe Bichard**, Michele Fior-Gozlan, Hubert Roth, Jean Auroux, Olivier Risse, Christian Letoublon, Marie Hélène Laverrière , Ivan Bricault, Vincent Leroy, Thomas Decaens

Endosc Int Open 4, E997–E1003 (2016).

Considering the encouraging results of publication1 we conducted a prospective study from 2007 to 2012 in patients with biliary stenosis and a suspected diagnosis of cholangiocarcinoma. In this study 111 consecutive patients with an indeterminate biliary stenosis were included with a final diagnosis of a malignant stenosis in 51 patients including 43 cholangiocarcinoma (84%). Patients with a stenosis due to an extrinsic compression identified on a previous imaging technique were excluded.

Results

- The sensitivity of brushing alone was 66.7%
- **The sensitivity of combination of bile aspiration before and after brushing was significantly better: 84.3% (p = 0.004) with a specificity of 100% and a diagnosis accuracy of 92.8%.**

Impact for ERCP practice

This study confirms that performing bile aspiration before and after brushing during ERCP significantly increased the ability to diagnose malignant bile-duct stenosis. This method could be used as a standard procedure to improve the diagnosis of malignant stenosis, especially cholangiocarcinoma.

Performance of bile aspiration plus brushing to diagnose malignant biliary strictures during endoscopic retrograde cholangiopancreatography

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submitted
11. December 2015
accepted after revision
17. May 2016

Bibliography
DOI http://dx.doi.org/10.1055/s-0042-108854
Published online: 9.8.2016
Endoscopy International Open 2016; 04: E997–E1003

E-ISSN 2196-9736

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Background and study aims: Endobiliary brushing during endoscopic retrograde cholangiopancreatography (ERCP) is the main technique used to diagnose a malignant stricture, but has a poor sensitivity.
This study evaluated the diagnostic performance of bile aspiration associated with biliary brushing during ERCP to diagnose a malignant stricture, compared to brushing alone.
Patients and methods: Between January 2007 and December 2012, all consecutive patients undergoing ERCP to treat a biliary stricture were included. After a biliary sphincterotomy, 3 mL to 10 mL of bile was aspirated into the brush catheter and collected in a dry sterile tube before and after brushing (to yield three samples). Brushing was performed as commonly recommended.
Results: One hundred eleven patients (68 males, 43 females) were included; mean age 67 ± 15.4 years. A final diagnosis of malignant stricture was established in 51 patients, including 43 cholangiocarcinomas; 60 patients had benign strictures. Specificity (Sp) and positive predictive values were 100% for all samples. The diagnostic performance of the three-sample combination of bile aspiration + brushing + bile aspiration was significantly greater than brushing alone (P = 0.004): sensitivity (Se) = 84.3 % vs. Se = 66.7 %. The three-sample combination gave a negative predictive value of 88.2 %, and a diagnostic accuracy of 92.8 %. When suspicious results were added to malignant results as positive results, the three-sample combination gave Sp = 91.7 % and Se = 94.1 %.
Conclusions: In cases of biliary stricture, conducting bile aspiration before and after brushing significantly increased the ability to diagnose a malignant stricture with a sensitivity of 84.3 % (P = 0.004).

Introduction

Cholangiocarcinoma is the second commonest primary malignancy of the liver; incidence and mortality rates are increasing worldwide [1,2]. At the time of diagnosis, most patients have an unresectable disease and the global 5-year survival rate is < 5 % [3].
Noninvasive techniques, such as magnetic-resonance cholangiopancreatography, have shown great sensitivity and specificity in detecting malignant biliary strictures [4,5] and are particularly efficient at evaluating the resectability of biliary tumors. Nonetheless, MRI does not provide any therapeutic action or anatomopathologic proof. Also, as fewer than 20 % of cholangiocarcinomas are suitable for surgical resection [1], biliary drainage using endoscopic retrograde cholangiopancreatography (ERCP) is often needed in cases of symptomatic biliary stenosis. Thus, ERCP represents a good opportunity to get an anatomopathologic diagnosis in order to guide the therapeutic approach.
Biliary brushing during ERCP was first described by Osnes et al. in 1975 [6] and is nowadays the most commonly used diagnostic technique in cases of symptomatic biliary stricture to provide both a diagnosis as well as being a first-line therapy. It is a safe and simple diagnostic technique [7,8] but its diagnostic performance is considered insufficient, as reported in three recent meta-analyses, with sensitivities between 41 % and 45 % [9–11]. With the aim of increasing diagnostic yield from biliary cytology, many studies have also evaluated new generations of cytobrush, but with disappointing results [12], or in limited series of patients [13].
The aim of our study was to evaluate the diagnostic accuracy of bile aspiration in association with biliary brushing used during ERCP. This three-

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sample combination was compared with brushing alone in a large prospective cohort of patients with a biliary stricture.

Patients and methods

Patients

This study was performed at the Universitary Hospital of Grenoble-Alpes, France, from January 1, 2007 to December 31, 2012. Every patient that underwent ERCP to treat a biliary stricture with an undetermined diagnosis was prospectively recruited. Every stenosis was confirmed from preoperative imaging studies.
Patients with a previous diagnosis of bile-duct malignancy were excluded. Stenoses caused by an extrinsic compression identified by preoperative imaging were also excluded. Stenoses with the suspicion of an intraductal tumor were included. If a stenosis was found isolated without any element of etiological orientation on preoperative examinations, it was considered as an undetermined stenosis. In cases of lithiasis extraction, patients were included only if a stenosis was revealed by post-extraction opacification; these stenoses were called post-lithiasis stenoses if no other diagnosis was found.
Groups of patients were defined according to the undetermined character or not of the stenosis, the necessity or not to perform a dilatation before the sampling procedure, and the presence or not of an intraductal prosthesis placed during a previous procedure without realization of the three-sample combination either because first ERCP was performed in another center, or during an emergency. Patients with prosthesis were only included if stenoses were considered as undetermined.
Every patient gave informed written consent. Our study was approved by an institutional review board and respected the principles of the Declaration of Helsinki.

Sampling technique

After a biliary sphincterotomy, a 0.035-inch guidewire was placed through the stenosis. A single-use cytology brush protected by an 8-Fr catheter (ref. FS-CB-1.5-S Cook®) was slipped onto the guide wire and positioned above the stricture. Initially, 3 mL to 10 mL of bile was aspirated into the brush catheter using a connected syringe and it was collected in a dry sterile tube. Brushing was then conducted using 10 passages in a to-and-fro motion across the stricture. After brushing, a second bile aspiration was conducted according to the same criteria as the first one. Thus, 3 samples were obtained from each patient: 2 aspirates and 1 from brushing. If the stenosis was considered too narrow by the operator, it was dilated using a biliary dilatation balloon between first aspiration and brushing.

Cytopathologic analysis

Classical cytological analyses (May Grumwald Griemsa, Papanicolaou stainings) were performed on both bile and brushing samples, and thin-layer cytology (ThinPrep® PreservCyt Solution) was done on brushing samples. For each sample, the cytologist determined cellularity according to four categories (insufficient, poor, medium, rich) and the types of cells found (malignant, suspicious; inflammatory; normal).
For each sample, a positive result was defined as the presence of malignant cells and to consider the 3-sample combination as positive, at least 1 sample among 3 had to provide malignant cells. Suspicious, inflammatory and normal samples were considered as negative. In addition, we then analyzed the diagnostic

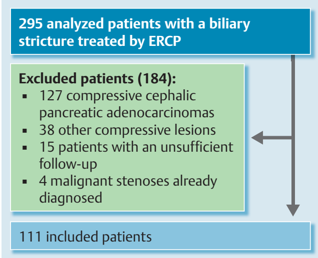

Fig. 1 Flowchart of 295 patients with biliary stricture plus per-ERCP three-sample combination. Data were collected between January 2007 and December 2012.

accuracy of each sample when suspicious results were added to malignant results as positive results.
Malignancy was confirmed by histopathologic analysis or using evidence of clinical and radiological progression during the follow-up period. A benign stricture was defined as a stricture without suggestive evolution of a malignancy during a 1-year minimum follow-up, which included clinical, radiological, and biological surveillance, or histopathological analyses.

Statistical analyses

We used the non-parametric Wilcoxon paired tests to compare the cellularity of the different samples from each patient. The sensitivity/specificity (Se/Sp) balance, according to the diagnosis obtained from each sampling method, was represented by area under ROC curves (AUC) (with 95 % CI), and paired analyses were used to compare the AUCs between the 3-sample combination and brushing alone (Stata's *roccomp* command for correlated data), with a Bonferroni correction for multiple tests when required. AUCs were also used to compare the diagnostic performances of each sample between the different groups of patients. When the AUC comparison was not adapted to assess if sensitivity was significantly increased or not because of variations in specificity, both sensitivity and specificity were compared on their respective sides, between different sampling techniques, using the McNemar test.
All statistical tests were 2-sided, and a P value < 0.05 was regarded as statistically significant. Statistical analyses were performed using IBM SPSS Statistics software, version 20.0 (IBM Corp., Armonk, NY), and Stata 13 (Stata Corp., College Station, TX).

Results

Characteristics of the patients

From January 1, 2007 to December 31, 2012, 295 patients with a biliary stricture underwent an ERCP. As shown in **Fig. 1**, 127 patients were excluded because of a compressive pancreatic adenocarcinoma, 38 patients because of other compressive lesions, 15 patients because of a too short follow-up, and 4 patients because a histologic diagnosis had already been obtained before the ERCP. Thus, a total of 111 consecutive patients were included in the analyses.
The patients' main characteristics are summarized in **Table 1**. There were 68 males and 43 females, with a mean age of 66.8 ± 15.4 years. Biliary stenoses were located in the common bile duct in 77 cases (69.4 %), on the bile-duct bifurcation in 27 cases (24.3 %), and 7 had intrahepatic strictures (6.3 %).
Seventy-six patients (68 %) presented with an undetermined stenosis and 16 of them were later diagnosed as being malignant

Table 1 Patient characteristics.

Population characteristics	Data
Age, mean (SD)	66.8 (15.4)
Gender, n (%)	
Male	68 (61 %)
Female	43 (39 %)
Stenosis localization, n (%)	
Common bile duct	77 (69.4 %)
Bile-duct bifurcation	27 (24.3 %)
Intra-hepatic ducts	7 (6.3 %)
Undetermined stenosis, n (%)	
Yes	76 (68 %)
No	35 (32 %)
Dilatation before brushing, n (%)	
Yes	24 (22 %)
No	87 (78 %)
Prosthesis from a previous ERCP, n (%)	
Yes	18 (16 %)
No	93 (84 %)
Post-ERCP adverse events, n (%)	9 (8.1 %)
Pancreatitis	3 (2.7 %)
Angiocholitis	3 (2.7 %)
Hemorrhage	2 (1.8 %)
Perforation	1 (0.9 %)

(22 %). In contrast, 35 (32 %) diagnoses of a tumoral stenosis were already suspected from preoperative imaging because of the presence of an intraductal process or metastasis. Among all the patients, there were 9 ERCPs (8.1 %) with adverse events (AEs) as described in **Table 1**.
A final diagnosis of malignant stricture was established in 51 patients with 7 pancreatic adenocarcinomas without any nodules, 43 cholangiocarcinomas and 1 ampulloma, and in 60 cases, the stenosis was considered benign as detailed in **Table 2**. Sixteen malignant stenoses and 10 benign stenoses were confirmed by histologic examination.

Cytology yield

All included ERCPs allowed all 3 samples to be obtained. As shown in **Fig. 2**, only 17 (15.3 %) brushing samples were rich in cells whereas bile aspiration produced samples with rich cellularity in 62 (55.9 %) cases before brushing and 66 (59.5 %) cases after brushing. The addition of medium-cellularity samples to the cell-rich samples, considered as "high-quality" samples, represented 74.8 % and 80.2 % of samples with bile aspiration before and after brushing, respectively, whereas brushing provided only 52.3 % of "high quality" samples. Consequently, bile aspiration provided better-quality samples than brushing alone ($P<0.0001$), without significant difference between both bile aspiration samples ($P=0.484$).

Diagnostic performances

The data on the diagnosis of malignant stenoses and the detection of malignant cells through cytology are shown in **Table 3**. Each sampling technique had a specificity (Sp) and a positive predictive value (PPV) of 100 %. The sensitivities (Se) and negative predictive values (NPVs) were as follows: 66.7 % and 77.9 % for brushing alone, 72.6 % and 81.1 % when bile aspiration was done before brushing, and 76.5 % and 83.3 % when bile aspiration was done after brushing. None of these results varied significantly from each other.

Table 2 Final diagnosis.

Type of stenosis	n (%)
Malignant strictures	51 (46 %)
Pancreatic adenocarcinoma	7 (6 %)
Cholangiocarcinoma	43 (39 %)
Peri-hilar tumor	24
Common bile duct	18
Intrahepatic	1
Ampullary carcinoma	1
Benign strictures	**60 (54 %)**
Pancreatitis	16 (14 %)
Acute pancreatitis	3
Chronic pancreatitis	13
Primary sclerosing cholangitis	10
Postoperative stenosis	10
Post-lithiasis stenosis	8
Odditis and other benign ampullary stenoses	7
Undetermined benign stenosis	9

Se and NPV of the 3-sample combination reached respectively 84.3 % and 88.2 %, with a diagnostic accuracy of 92.8 % which was significantly superior to brushing alone ($P=0.004$).
Combining brushing with only 1 bile-aspiration sample as a way to eventually increase the cost-effectiveness of the technique still gave significantly higher results than brushing alone, as shown in **Table 3**, with the same ability to diagnose a malignant stenosis as the 3-sample combination, for the association of brushing plus bile aspiration after brushing.
When suspicious cells in addition to malignant cells were considered as positive results (**Table 4**), brushing alone was able to diagnose malignant stenoses at Sp = 95 % and Se = 80.4 %, whereas the 3-sample combination had significantly greater sensitivity (Se = 94.1 %, McNemar test: $P=0.008$), without a significant decrease in specificity (Sp = 91.7 %, McNemar test: $P=0.157$).

Group analyses

Data for undetermined stenosis (n = 76) are summarized in **Table 5**. Sp and PPV were still 100 %, and Se and NPV were as follows: brushing alone, 43.8 % and 87.0 %, respectively, and 3-sample combination, 62.5 % and 90.9 %, respectively. No statistical differences were found ($P=0.06$). When suspicious cytologic results were combined with malignant cells, brushing alone resulted in Sp of 95.0 % and Se of 68.8 % whereas the 3-sample combination resulted in Sp of 91.7 % and Se of 87.5 % (**Table 6**). No statistical differences were found (AUCs comparison: $P=0.136$; McNemar test: $P=0.0833$).
In patients with a biliary dilatation before sampling (n = 24), diagnostic accuracy was unchanged for brushing, and increased for the 3-sample combination with sensitivity of 100 % ($P=0.002$) (**Table 7**).
In patients with a prosthesis from a previous procedure (n = 18), diagnostic accuracy was dramatically decreased with a sensitivity of only 40 % for both brushing alone and the 3-sample combination (**Table 8**).

Discussion

This study shows that the combination of bile aspiration and brushing increased diagnostic yield and gave richer cellularity, and thus, greater diagnostic accuracy from cytologic analyses

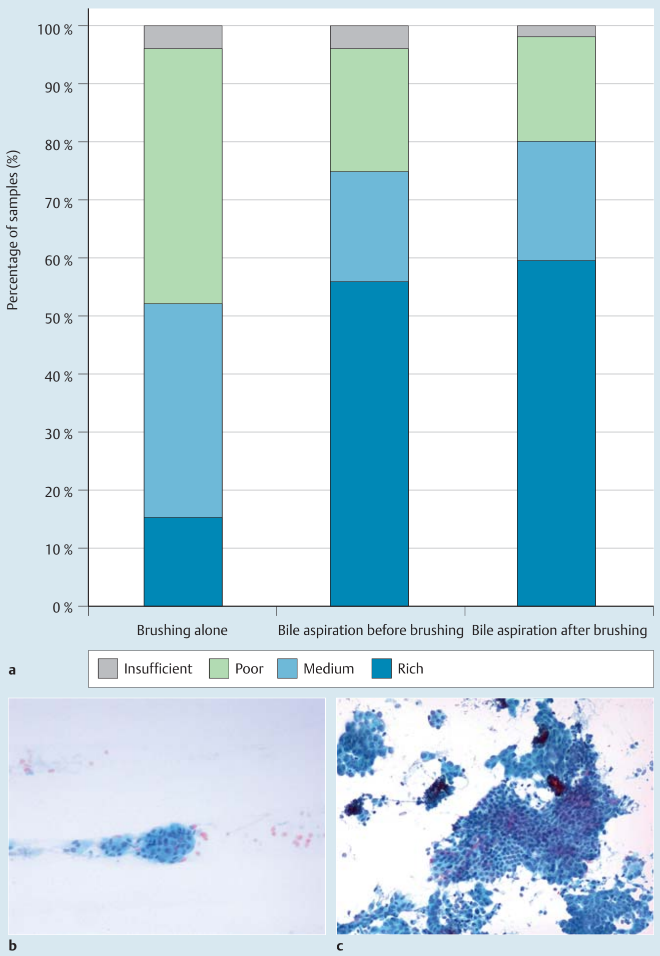

Fig. 2 **a** Cellularity within the cytologic samples: percentages of rich, medium, poor and insufficient cells per sampling technique. **b** Pictures illustrating cell quantity with Papanicolaou staining and magnification × 10 of a sample obtained by brushing. **c** Pictures illustrating cell quantity with bile aspiration.

Table 3 Accuracy of diagnosis of malignancy after detection of malignant cells according to the different sampling methods.

Detection of malignant cells	Sp (%)	Se (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)	AUC [95 % CI]	Bonferroni *P* value
Brushing alone	100	66.7	100	77.9	84.7	0.833 [0.768:0.899]	Reference
Bile aspiration before brushing	100	72.6	100	81.1	87.4	0.863 [0.786:0.940]	1
Bile aspiration after brushing	100	76.5	100	83.3	89.2	0.882 [0.824:0.941]	0.646
Three-sample combination	100	84.3	100	88.2	92.8	0.922 [0.871:0.972]	0.004
Combinations of two samples							
Bile aspiration and then brushing	100	78.4	100	84.5	89.2	0.892 [0.823:0.922]	0.039
Brushing and then bile aspiration	100	84.3	100	88.2	92.8	0.922 [0.871:0.972]	0.004

Sp, specificity; Se, sensitivity; PPV, positive predictive value; NPV, negative predictive value.

Table 4 Accuracy of diagnosis of malignancy after detection of malignant and suspicious cells with different sampling methods.

Detection of malignant and suspicious cells	Sp (%)	Se (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)	AUC [95 % CI][1]	*P* value
Brushing alone	95.0	80.4	93.2	85.1	88.3	0.877 [0.804:0.950]	Reference
Bile aspiration before brushing	95.0	84.3	93.5	87.7	90.0	0.897 [0.829:0.964]	ND
Bile aspiration after brushing	93.3	86.3	91.7	88.9	90.2	0.898 [0.832:0.964]	ND
Three-sample combination	91.7	94.1	90.6	94.8	92.8	0.945 [0.895:0.994]	0.054

Sp, specificity; Se, sensitivity; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.
ND = not determined because of irrelevance of this statistical comparison and the increase of alpha error if done.
[1] AUCs were compared between "Brushing alone" and the "Three combined samples".

Table 5 Accuracy of diagnosis of malignancy after detection of malignant cells with different sampling methods for undetermined stenoses.

Detection of malignant cells	Sp (%)	Se (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)	AUC [95 % CI][1]	*P* value
Brushing	100	43.8	100	87.0	88.2	0.719 [0.593:0.844]	Reference
Bile aspiration before brushing	100	43.8	100	87.0	88.2		ND
Bile aspiration after brushing	100	50.0	100	88.2	89.5		ND
Three-sample combination	100	62.5	100	90.9	92.1	0.813 [0.690:0.935]	0.063

Sp, specificity; Se, sensitivity; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.
ND = not determined because of irrelevance of this statistical comparison and the increase of alpha error if done.
[1] AUCs were compared between „Brushing alone" and the "Three combined samples".

Table 6 Accuracy of diagnosis of malignancy after detection of malignant and suspicious cells with different sampling methods for undetermined stenoses.

Detection of suspicious and malignant cells	Sp (%)	Se (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)	AUC [95 % CI][1]	*P* value
Brushing	95.0	68.8	78.6	91.9	89.5	0.819 [0.698:0.939]	Reference
Bile aspiration before brushing	95.0	68.8	78.6	91.9	89.5		ND
Bile aspiration after brushing	93.3	68.8	73.3	91.8	88.2		ND
Three-sample combination	91.7	87.5	73.7	96.5	90.8	0.896 [0.805:0.987]	0.136

Sp, specificity; Se, sensitivity; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.
ND = not determined because of irrelevance of this statistical comparison and the increase of alpha error if done.

Table 7 Detection of malignant cells with or without dilatation before brushing.

Detection of malignant cells	Dilatation (Yes/No)	Sp (%)	Se (%)	PPV (%)	NPV (%)	AUC [95 % CI]	*P* value
Brushing	No	100	66.7	100	75.0	0.833 [0.758:0.908]	Reference
	Yes	100	66.7	100	78.7	0.833 [0.694:0.973]	1.000
Bile aspiration before brushing	No	100	66.7	100	78.7	0.833 [0.758:0.908]	Reference
	Yes	100	91.7	100	92.3	0.958 [0.877:1.000]	0.027
Bile aspiration after brushing	No	100	71.8	100	81.4	0.859 [0.787:0.931]	Reference
	Yes	100	91.7	100	92.3	0.958 [0.877:1.000]	0.073
Three-sample combination	No	100	83.7	100	85.7	0.897 [0.833:0.962]	Reference
	Yes	100	100	100	100	1.000 [1.000:1.000]	0.002

Sp, specificity; Se, sensitivity; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.

[1] AUCs were compared between "Brushing alone" and the "Three combined samples".

Table 8 Detection of malignant cells with or without a prosthesis before ERCP.

Detection of malignant cells	Prosthesis (Yes/No)	Sp (%)	Se (%)	PPV (%)	NPV (%)	AUC [95 % CI]	*P* value
Brushing	No	100	69.6	100	77.0	0.847 [0.781:0.915]	Reference
	Yes	100	40.0	100	81.2	0.700 [0.460:0.940]	0.245
Bile aspiration before brushing	No	100	78.7	100	82.5	0.891 [0.831:0.952]	Reference
	Yes	100	20.0	100	76.5	0.600 [0.404:0.796]	0.005
Bile aspiration after brushing	No	100	80.4	100	83.9	0.902 [0.844:0.960]	Reference
	Yes	100	40.0	100	81.2	0.700 [0.460:0.940]	0.109
Three-sample combination	No	100	89.1	100	90.4	0.945 [0.900:0.991]	Reference
	Yes	100	40.0	100	81.2	0.700 [0.460:0.940]	0.049

Sp, specificity; Se, sensitivity; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.

compared to brushing alone. Indeed, by providing a large amount of cells, bile aspiration increases the probability of detecting tumor cells. Brushing has a low yield and is often insufficient to establish a clear diagnosis, but none of the bile samples alone had statistically greater diagnostic accuracy than brushing. Thus, there is no argument for excluding brushing from the standard procedure, and it has to be associated with at least 1 bile sample, especially bile-aspiration after brushing whose association reaches the same rate of diagnosis to detect malignant cells as the 3-sample combination (Se = 84.3 %, Sp = 100 %). Brushing may potentiate the cytologic yield from bile aspiration conducted after brushing by increasing desquamation of bile-duct cells through its mechanical action, even if we did not find statistical difference between bile aspiration before and after brushing. Also, good performance of brushing alone could be due, in part, to an increase in cells trapped in the brush during first bile aspiration.

In this study, we first tested malignant cells only, to obtain a positive-diagnosis result, and excluded suspicious results from the analyses. However**,** we then tested the diagnostic performances of each of the 3 different samples individually and in combination when suspicious results were included in the positive results. The 3-sample combination showed very high sensitivity (Se = 94.1 %), which was statistically superior to brushing alone (P = 0.008) and did not significantly decrease specificity (Sp = 91.7 %). This confirms the results of a previous study, conducted in 2012, which assessed bile aspiration in 42 patients with a suspected malignant biliary stricture and found a sensitivity of 89 % to detect malignant stenosis when combining suspicious and malignant cells [14].

ERCP is often done after determinant imaging, such as computed tomography scans or magnetic resonance imaging. Consequently, in our study, the majority of malignant biliary stenoses were already suspected after the preoperative work-up. Nevertheless, we wanted to analyze the diagnostic performance of our technique in cases of biliary stenoses when imaging could not provide a suspected diagnosis, which corresponds to a classical but difficult situation for clinicians. In these undetermined stenoses, a diagnosis of malignancy within each sample remained low, without a significant difference between brushing alone and the 3-sample combination, Se = 43.8 % vs. 62.5 % (= 0.063), probably because of a lack of statistical power. The addition of suspicious results to positive results revealed a sensitivity to diagnose malignant stenoses of almost 90 % in undetermined stenoses when the 3 samples were combined, with only 2 false-negative results (one ampullary adenocarcinoma diagnosed 1 year later, and 1 cholangiocarcinoma), whereas the sensitivity of brushing alone remained inferior to 70 % (P = 0.083). Even if this did not reach statistical difference, it showed again how much bile aspiration seems to help the detection of malignant stenoses. The addition of suspicious cells could increase the risk of unnecessary surgical procedure. However, as mentioned before, realization of ERCP is always included in a clinical context with additional examinations which can guide the strategy. Moreover, avoid a false-negative result is also a major issue regarding the severity of biliary malignant diseases. Thus, the treatment of patients with suspicious cells without malignant cells has to be discussed and requires a multidisciplinary decision.

As the presence of an endobiliary prosthesis dramatically decreased the sensitivity of all samples, it seems essential to assess the 3-sample combination before any prosthesis placement to increase the chance of achieving a cytologic diagnosis. In addition, our new method had an overall AE rate that was comparable to large series in the literature [7, 8, 15].

One of the limits of our study is its monocentric nature. Indeed cytologic diagnosis has interobserver variation [16]. Because the vast majority of the samples were analyzed by the same pathologist, the result of this monocentric study has to be confirmed by a multicenter prospective study comparing the brushing alone to the 3-sample combination. A bigger study would also increase the statistical power, which is limited especially for undetermined stenosis. Another limit is the absence of blind cytologic analysis. Nevertheless, in all undetermined stenoses, cytologists were, by definition, blinded, and diagnostic performances of bile aspiration were still much better than those of the brushing.

In our study, only a few patients had a histopathologic confirmation for obvious ethical reasons. In the large majority of patients, the diagnostic confirmation was based on their clinical course in order to confirm or exclude the possibility of a malignant stenosis, as described in the method section. This attitude corresponds to the present standards of clinical practice in front of a biliary stenosis.

By its greater sensitivity to diagnose malignant biliary stenosis, it should reduce the number of unnecessary additional diagnostic procedures and the overall cost of diagnosing biliary strictures, even though cost-effectiveness was not investigated. Moreover, this technique can be used by all biliary endoscopists who perform ERCP, as its technical difficulty is the same as for brushing alone. Our method seemed to have a much higher diagnostic yield than another simple and routinely used technique, such as per ERCP intraductal biopsies, which had a sensitivity of 48.1 % in a recent meta-analysis [11]. Also, several studies report on methods attempting to improve diagnostic yield from endobiliary samples through researching new biological markers. For example, fluorescent in situ hybridization seems to have very high diagnostic performances (Se = 89 % and Sp = 97 %) when detection of deletion 9q21 and polysomy is associated with cytologic analysis of brushing [17]. These diagnostic performances could even be better in association with our new method. Also, some new markers are assessed in bile such as neutrophil gelatinase-associated lipocalin (NGAL) with promising results in the diagnosis of malignant stenosis, which suggested that bile is very interesting to study [18]. On the other hand, new endoscopic techniques such as cholangioscopy biopsies and confocal laser endomicroscopy are being developed. The first one suffers from a low sensitivity, around 75 % [19]. The second one requires a really high level of expertise, and despite its excellent sensitivity, its diagnostic performance is still low as its specificity is 67 % in a recent prospective study [20]. For now, none of these techniques seem to have a greater diagnostic accuracy and they are costly procedures which are not easily accessible to the majority of endoscopic centers.
In conclusion, our study shows that performing bile aspiration before and after brushing during ERCP significantly increased the ability to diagnose malignant bile-duct strictures. This method could be used as a standard procedure to improve the diagnosis of malignant strictures, especially cholangiocarcinoma.

Competing interests: None

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5 Publication 3 [57]

Impact of peroral cholangioscopy on the management of indeterminate biliary conditions: a multicentre prospective trial.

Prat F, Leblanc S, Foissac F, Ponchon T, Laugier R, **Bichard P**, Maire F, Coumaros D, Charachon A, Vedrenne B, Boytchev I, Chaussade S, Kaddour N, Laquière A, Gaujoux S

Frontline Gastroenterol. 2019 Jul;10(3):236-243.

In this prospective, multicenter study we included 61 patients from nine tertiary centers with PSC (n = 13) or indeterminate biliary stenosis (n = 48). Indeterminate biliary stenosis was strictly defined: no nodule justifying an EUS-FNA was present, no history suggested a definitive diagnosis and at least one ERCP with brushing and / or fluoroscopically guided biopsies was inconclusive. Primary endpoint was the difference in adequacy of management planned before and after single-operator cholangioscopy (SOC).

Results

- The management adequacy rate was significantly higher after SOC than before SOC overall ($p<10^{-5}$), in indeterminate biliary stenosis ($p<0.001$) and PSC ($p<0.05$) patients.
- **SOC induced changes in the management of the majority of patients in 60.3% of PSC and 58% of indeterminate biliary stenosis**
- The specificity, the positive and negative predictive values of SOC were respectively 100%, 100% and 83.6%

Impact for ERCP practice

This study suggests that SOC induces changes in management and avoids unnecessary surgery in the majority of patients with indeterminate biliary stenosis and PSC. SOC should be used in the assessment of selected patients during PSC or indeterminate biliary stenosis with a suspicion of malignancy. Especially when there is a significant impact on attitude: surgical resection vs conservative management

ORIGINAL ARTICLE

Impact of peroral cholangioscopy on the management of indeterminate biliary conditions: a multicentre prospective trial

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/flgastro-2018-100985).

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Received 9 March 2018
Revised 10 October 2018
Accepted 18 October 2018
Published Online First 27 November 2018

To cite: Prat F, Leblanc S, Foissac F, *et al*. *Frontline Gastroenterology* 2019;**10**:236–243.

ABSTRACT

Background and aims Single-operator cholangioscopy (SOC) can help diagnose biliopancreatic conditions. The impact of SOC on patient outcome has never been specifically addressed.

Patients and methods Consecutive patients bearing indeterminate biliary strictures (IDBS), or primary sclerosing cholangitis (PSC) with suspected cholangiocarcinoma, were included. Patients with IDBS had at least one previous inconclusive endoscopic retrograde cholangio pancreatography (ERCP) + cytology. Primary endpoint was the difference in adequacy of management planned before and after SOC with regard to final diagnosis obtained after surgery or 24 months follow-up.

Design Prospective open-label multicentre trial.

Results 61 patients were included (IDBS: 48; PSC: 13); 70.5% had a benign lesion (IDBS 66.7%, PSC 84.6%). The management adequacy rate was significantly higher after SOC than before SOC overall ($p<10^{-5}$), in IDBS ($p<0.001$) and PSC ($p<0.05$) patients. SOC induced changes in the management of the majority of patients in all groups (60.3%). The overall sensitivity of combined visual impression and biopsy ranged from 52% to 63.6% depending on investigator or independent expert rating (κ 0.92–0.96), whereas specificity, positive and negative predictive values of SOC were, respectively, 100%, 100% and 83.6%. Patient management observed at the end of follow-up was consistent with that anticipated after SOC in 88.5% overall.

Conclusion Despite a moderate sensitivity for the diagnosis of malignancy, SOC has a dramatic impact on the management of patients with IDBS and PSC with suspected carcinoma. Cholangioscopy might be implemented in the workup of selected patients with challenging diagnosis, when a significant impact on outcome (essentially resection vs conservative management) is to be expected.

INTRODUCTION

A majority of biliary strictures are malignant, mostly due to the extrinsic compression of the distal bile duct by a pancreatic head cancer, less commonly to the intrinsic development of a cholangiocarcinoma or to the ductal extension of a gallbladder carcinoma or lymphatic metastases from a distant tumour. Benign biliary strictures, accounting for up to 30% of cases,[1] often have an obvious cause, be it a swollen pancreatic head during the course of chronic pancreatitis, an ischaemic duct-to-duct anastomosis after liver transplantation, sequelae of bile duct injury within months of a cholecystectomy, and so on. However, the standard workup of a biliary stenosis, including blood tests, abdominal ultrasound, CT scan, MRI and endoscopic ultrasonography (guided) - fine needle aspiration (EUS-FNA), fails in some patients to identify the aetiology of the stricture, dictating the need to perform ERCP with cytology. Due to the poor cytological yield of ERCP techniques (including bile and brush cytology and fluoroscopy-guided biopsies, which even in combination, barely reach 50% sensitivity),[2 3] a substantial contingent of strictures remain indeterminate. It is recognised that 5%–24% of patients undergoing surgery for suspected but not preoperatively proven malignancies do indeed have a benign condition and could have been spared such complex and sometimes high-risk operations.[4 5] It is therefore highly desirable to investigate more accurately so-defined indeterminate biliary strictures (IDBS) in order to reduce the rate of unnecessary surgeries and apply a treatment fit to the patient's condition. Primary sclerosing cholangitis (PSC) may raise similarly difficult diagnostic issues.[6] Cholangioscopy, as a method with an ability to visualise strictures endoscopically and to target biopsies, has been proposed to further advance investigation in such difficult cases, particularly after single-use, single-operator devices were made available in 2007.

Several studies found single-operator cholangioscopy (SOC) to bear high-sensitivity and negative predictive value for the diagnosis of malignancy in case of IDBS and PSC. However, most of those works, later discussed in this article, did not study the impact of SOC on the patient's outcome. It was the primary aim of the present study to assess to what extent SOC could affect patient management in case of IDBS and PSC with suspected cholangiocarcinoma.

PATIENTS AND METHODS

The scope of this study encompassed two categories of biliary conditions in which to assess the impact of SOC, namely IDBS and PSC. Over a 3-year period, patients from nine French academic tertiary referral centres[i] fulfilling selection criteria were included, then prospectively followed during 24 months. Selection criteria were adapted for each of the three subgroups. A definitive diagnosis was obtained either after surgical resection or after follow-up. Observed managements and outcomes after SOC were compared for each patient to the initially planned management (ie, before SOC) as well as to currently recommended management after final observations were disclosed, under the authority of independent experts.

Patient selection

Patients were aged >18 years, were covered by social security insurance and signed informed consent of participation. All the patients had undergone a standard workup including biology, CT scan or MRI, EUS with or without FNA. All patients were rated I or II under the American Society of Anesthesiology classification and deemed fit for surgery. In the IDBS group, only patients with clinically overt strictures (ie, with biological cholestasis and/or jaundice, with or without clinical symptoms of biliary obstruction) were included. Strictures could involve the common bile duct, the main confluence/hilum and/or the peri-hilar part of major intrahepatic ducts. At least one ERCP with inconclusive brush cytology[ii] was required for the stricture to be deemed as IDBS. The last ERCP had to have been performed <4 weeks before inclusion. Patients with a conclusive array of aetiological factors (ie, orthotopic liver transplantation (OLT) patients with anastomotic stricture developed <1 year before transplantation, recently complicated biliary surgery, and swollen and compressive chronic pancreatitis) as well as those with a pancreatic head or lymphatic mass amenable to EUS-FNA (even after a first inconclusive FNA) were excluded. In the PSC group, patients had to present one of the following criteria: (a) a 'dominant' bile duct stricture, defined as a stricture comprised between the sectoral confluences and the papilla, longer than 5 mm, with upward ductal dilation; (b) multi disciplinary team meeting (MDT) request for exclusion of cholangiocarcinoma before OLT waiting list registration; and (c) newly observed Ca19-9 serum elevation above 130 UI/mL with or without weight loss (figure 1).

SOC procedure, diagnostic workup and follow-up

The contemplated management strategy, would SOC be inconclusive or provide no additional information, had to be consigned at inclusion and before SOC. Procedures were performed in supine position under general anaesthesia with airway intubation, using a standard large channel duodenoscope and the Spyglass-Legacy platform (Boston Scientific, Marlborough, Massachusetts, USA). Most patients had a previous endoscopic sphincterotomy, which could be enlarged at the time of SOC when needed. Cholangioscopy was done under saline irrigation and all patients received prophylactic antibiotics. SOC procedure always included an observational phase, with visual impression being described with as much detail as possible, although no specific terminology was required. It was however required to classify the visual impression as malignant, benign or still indeterminate. Intraductal targeted biopsies were obtained using the Spybite (Boston Scientific) miniforceps as often as possible, with at least four macroscopically visible samples, although it was accepted that no biopsy was taken when visual impression was strictly normal (ie, absence of surface or colour anomalies). Since all participating centres had their own pathology department with expertise in biliopancreatic diseases, pathological sample analyses were not centralised, but toughest cases were discussed, when necessary, with the study's referee pathologist (BT). A plastic stent was generally, but not systematically, inserted after removing the cholangioscope. The investigator was required to report whether their visual impression was likely or not to change the previously planned management. Patient management decided after SOC

[i]All tertiary referral endoscopy centres performed >400 ERCPs per year.

[ii]Inconclusive cytology=acellular, inflammatory cells, atypia. The presence of high-grade dysplasia was considered suggestive of neoplasia, and thus conclusive.

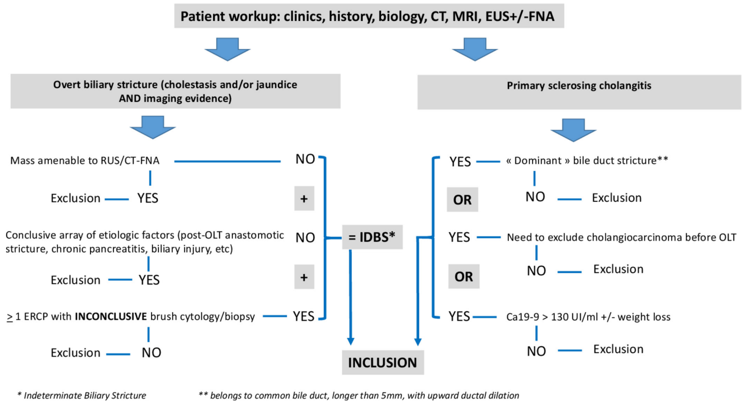

Figure 1 Diagram of selection criteria for patient enrolment. IDBS, indeterminate biliary strictures.

and pathology results by the investigator and/or during MDT meetings were recorded. SOC-induced changes within a given type of management (eg, a different type of resection, suggested by the observed extent of lesions) were also reported. Patients were followed after SOC until surgery in case of resection or during 24 months in case of conservative management, with visits scheduled at 3, 6, 9, 12, 18 and 24 months after SOC, including clinical examination, liver blood tests and imaging when needed. Definitive diagnosis and observed management were reported at the end of FU.

Objectives and endpoints

The main objective of the study was to determine the percentage of patients in whom SOC would change outcomes in a favourable manner, that is, by providing the evidence needed to make ad hoc decisions on patient management (ie, essentially surgical vs conservative). To achieve that goal, two endpoints were defined, for which the patient was his own control: (a) planned patient management before SOC versus management recommended after definitive diagnosis and (b) planned patient management after SOC versus management recommended after definitive diagnosis.

The following criteria defined the adequacy between diagnosis and management, depending on the subgroup of biliopancreatic condition: (a) In cases of IDBS, a benign stricture had to be treated by instrumental means, endoscopic or percutaneous, including dilations and (removable) stents, whereas malignant lesions should come under carcinologic resection, with explorations expected to define the extent of lesions to help the surgeon optimise the resection strategy; and (b) In the PSC group, patients with evidence of cholangiocarcinoma should have been excluded from transplantation and instead treated by oncological therapy adapted to their case, whereas transplantation could be confirmed, if needed, or conservative therapy be resumed, when carcinoma was precluded.

Secondary endpoints were (a) diagnostic performances of SOC, for which combined visual impression and pathology results were taken into account[iii]; (b) comparison of actually observed management *vs* management suggested after SOC; (c) comparison of actually observed management versus currently recommended management with regard to definitive diagnosis; and (d) proportion of patients in whom SOC has modified patients' management. Procedure duration and side effects were also reported.

The above-described adequacy endpoints (planned management after ERCP or SOC vs definitive diagnosis, actually observed management vs post-SOC suggested management) and classification of true and false negatives and positives for diagnostic performance were first assessed jointly by the principal investigators (FP, SL) for each individual patient. The same analysis was done subsequently from the same database by two experts blinded to the investigators' assessment. Those two assessors (one gastroenterologist (AL) and one surgeon (SG)), selected for their expertise in biliopancreatic disorders only after the end of data collection, were independent from the participating centres.

Number of subjects and statistics

Sample size calculation, with 80% power and a 5% significance level and discordant proportion of 50%, resulted in a total of 50 patients (25 patients per

[iii]Visual impression was deemed sufficient when suggesting a benign stenosis, that is, with no colour or surface anomaly; presence of malignant cells or high-grade dysplasia was required, whatever the visual impression, to diagnose malignancy; a visual impression of malignancy with no malignant or dysplastic cells, as well as cellular atypia with benign or malignant visual impression, were considered indeterminate.

subgroup). The calculation was based on an expected adequate management (with respect to definitive diagnosis) before SOC of 40% versus 80% after SOC.

Statistical analyses were performed using ad hoc routines implemented in the R V.3.3.1 software (http://www.r-project.org). Discrete variables are presented as counts (percentage) and continuous variables as mean ±SD for normally distributed variables and median (range) for those that did not have a normal distribution. Comparisons of the adequacy between planned management and definitive diagnosis before and after SOC were performed using McNemar tests for paired data. The inter-rater reliability (concordance between separate evaluators) regarding the classification of true and false negatives and positives for diagnostic performance was assessed by the Cohen's kappa coefficient using the PSY package.[7] Concordance was evaluated as follows: no agreement if <0, slight if 0–0.20, fair if 0.21–0.40, moderate if 0.41–0.60, substantial if 0.61–0.80 and almost perfect if 0.81–1.[8] A bootstrap method was used to calculate the 95% CI of the kappa's coefficients. All tests were two-sided at the 5% level of significance.

RESULTS

Sixty-seven patients were included overall, but six were excluded after two early non-procedure-related deaths 2 weeks and 2 months after inclusion and four patients lost at 24-month FU. Sixty-one patients, aged 58.7±14.6 years (median 61, range 21–89, with a sex ratio of 1.34), were analysed. In total, 48 belonged to the IDBS group (21 women, 27 men, aged 64.5±14 years) and 13 to the PSC group (5 women, 8 men, 48.2±16.7 years). There was no failure of cholangioscope insertion; balloon dilatation (up to 6 mm in diameter) was needed in two cases and repeat sphincterotomy in one case to allow for easy passage of the device. Exploration of the biliary tract above the stricture was possible in 77% (37/48) of patients in the IDBS group and 85% (11/13) in the PSC group. Quality of vision was deemed excellent/satisfactory in 85% of SOC procedures, fair or poor in 15%. Spybite biopsies were obtained from 57 patients, whereas 4 with cholangioscopically normal ducts had no biopsies, including 2 patients with unexpected bile duct stones. Two procedure-related cases of cholangitis (3.2%) occurred and resolved with antibiotics. The recorded time of cholangiopancreatoscopy per se (from Spyglass insertion to removal) was 31.1±1.4 min (median 25 min, range 5–90). The definitive diagnosis after surgery or 24-month FU was a benign disease in 43 patients (70.5%) and a malignancy in 18 (29.5%), as shown and broken down per group in table 1.

Table 2 shows that the adequacy between patient management as anticipated by investigators and the definitive diagnosis was significantly higher after SOC was performed than before, both for the overall casemix (p<0.0001), in patients with IDBS (p<0.001) and, although less significantly, in patients with PSC (p<0.05).

This is reflected in the rate of changes in planned management after SOC, which was estimated between 58.3% in patients with IDBS and 69.2% in patients with PSC (table 3).

Agreement between PIs and independent experts was perfect for this endpoint in all groups and overall (κ=1). Since most SOC procedures (57 out of 61) had been undertaken with an intent to operate for resection in case malignancy was confirmed, surgery was precluded after SOC in 58% of patients (33/57). More specifically, in patients with IDBS, surgery was confirmed as initially planned in 20/48 patients whereas 'active' management was changed to conservative in 26 and only 2 were changed to a more active treatment (surgery in one patient and chemotherapy in another). In patients with PSC, SOC has allowed to

Table 1 Relative numbers and proportions of benign and malignant diseases overall and per subgroup after definitive diagnosis was obtained (ie, after surgery or 24 months follow-up)

	Overall		Indeterminate biliary strictures		Primary sclerosing cholangitis	
	N	%	N	%	N	%
Benign	43	70.5	32	66.7	11	84,6
Malignant	18	29.5	16	33.3	2	15,4
Total	61	100	48	100	13	100

Table 2 Comparison of the adequacy between planned management and definitive diagnosis before and after single-operator cholangioscopy (SOC) for (a) all groups, (b) indeterminate biliary strictures (IDBS) and (c) primary sclerosing cholangitis (PSC)

(a) Overall ($p<10^{-5}$)	After SOC (%)		
	Inadequate	Adequate	
Before SOC (%)			
Inadequate	6	32	38 (62.3)
Adequate	5	18	23 (37.7)
	11 (18.0)	50 (82.0)	61 (100)

(b) IDBS (p<0.001)	After SOC (%)		
	Inadequate	Adequate	
Before SOC (%)			
Inadequate	3	24	27 (43.8)
Adequate	4	17	21 (56.2)
	7 (14.6)	41 (85.4)	48 (100)

(c) PSC (p<0.05)	After SOC (%)		
	Inadequate	Adequate	
Before SOC (%)			
Inadequate	3	8	11 (84.6)
Adequate	1	1	2 (15.4)
	4 (30.8)	9 (69.2)	13 (100)

Table 3 Single-operator cholangioscopy-induced changes in planned management

Planned management modified	Overall		Indeterminate biliary strictures		Primary sclerosing cholangitis	
	N	%	N	%	N	%
No	24	39.3	20	41.7	4	30.8
Yes	37	60.7	28	58.3	9	69.2
Total	61	100	48	100	13	100

confirm the registration of three patients on the OLT waiting list and exclude one other from prospective OLT, whereas seven others were avoided unnecessary hepatobiliary resection.

Diagnostic performance results (table 4) have been presented only for the overall casemix and for IDBS group because the small size of the PSC group, with very large CIs, made a correct interpretation of results uncertain (data available as online supplementary material). The overall and IDBS results demonstrate

Table 4 Diagnostic performance of single-operator cholangioscopy (SOC) (overall and indeterminate biliary strictures (IDBS))*

Performance criteria (95% CI)	Overall	IDBS
(a) SOC visual impression (PI)		
Se	63.6 (40.7 to 82.8)	64.7 (38.3 to 85.8)
Sp	100 (93 to 100)	100 (88.8 to 100)
PPV	100 (76.8 to 100)	100 (71.5 to 100)
NPV	86.4 (75 to 94)	83.8 (68.0 to 93.8)
(b) SOC visual impression + pathology (PI)		
Se	54.5 (32.2 to 75.6)	56.3 (29.9 to 80.2)
Sp	100 (93 to 100)	100 (89.1 to 100)
PPV	100 (73.5 to 100)	100 (66.4 to 100)
NPV	83.6 (71.9 to 91.8)	82.1 (66.5 to 92.5)
(c) Expert review 1 (AL) for SOC visual impression + pathology		
Kappa (CI95%) PI vs AL overall: 0.94 (0.84–1.0)/IDBS: 0.92 (0.80–1.00)		
Se	57.1 (34.0 to 78.2)	56.3 (29.9 to 80.2)
Sp	100 (93 to 100)	100 (89.1 to 100)
PPV	100 (73.5 to 100)	100 (66.4 to 100)
NPV	85.0 (73.4 to 92.9)	82.1 (66.5 to 92.5)
(d) Expert review 2 (SG) SOC visual impression + pathology		
Kappa (CI95%) PI vs SG overall: 0.97 (0.90–1.0)/IDBS: 0.96 (0.86–100)		
Se	52.0 (29.8 to 74.3)	50.0 (24.7 to 75.3)
Sp	100 (93 to 100)	100 (89.1 to 100)
PPV	100 (71.5 to 100)	100 (63.1 to 100)
NPV	83.6 (71.9 to 91.8)	80 (64.4 to 90.9)

*Indeterminate visual impression is classified as negative for malignancy.
PI, classification by principal investigators (SL, FP).
NPV, negative predictive value; PPV, positive predictive value; SE, sensitivity; SP, specificity

high specificity and positive predictive values of SOC at 100%, high negative predictive value but moderate sensitivity, the latter being slightly higher when visual impression is considered than when pathology results are introduced. Although up to five instances of disagreement in classification between principal investigator (PI) and experts have been noted, kappa agreement indices remained in the near perfect range at >0.88.

Finally, we tried to determine whether the actually observed management was in conformity with what SOC results suggested (table 5).

In seven patients (11.4%) overall the actual treatment was not found in accordance with SOC findings. These inconsistencies resulted from an error in cholangioscopic interpretation or false negative biopsy, an unexpected change in the patient's clinical course or a decision from the patient's or the doctor's part independently from objective findings. Details of each case are presented as online supplementary material.

DISCUSSION

In this study, we show that not only SOC improves the diagnosis of biliopancreatic lesions compared with ERCP with brush cytology—a previously accepted notion, especially for PSC and IDBS,[9–12] but we also show for the first time that SOC can favourably change disease management in a large proportion of patients. The diagnostic workup of biliary disease is straightforward when a mass, generally a malignant one, is amenable to EUS-FNA, or when history and imaging are typical of a specific aetiology, often a 'benign' one. Much more difficult is diagnosis when such a presentation is lacking. In the case of PSC, the identification of a malignant stricture among many others, inflammatory ones, is particularly challenging.

More than 10 years ago, peroral cholangioscopy had been shown to improve diagnostic performance in patients with IDBS or PSC with a dominant stricture.[9 13] In the landmark study by Fukuda *et al*, sensitivity for malignancy compared with ERCP + brush cytology increased from 58% to 100% and accuracy from 78% to 93% in a series including 38 cholangiocarcinomas and 38 benign lesions.[13] These early works were achieved with 'mother and baby' systems using a reprocessable cholangioscope, a technique which did not reach wide acceptance for at least four reasons, namely the high cost of the investment in a cholangioscope for a limited number of uses, the brittleness of the device with high repair costs, the need for two operators and the non-sterile device to be introduced in a supposedly sterile cavity. The advent of SOC in 2007 has made cholangioscopy a much easier, although still costly procedure, with a single-use sterile device, operated by a single endoscopist and requiring a more affordable investment in a relatively low-tech endoscopy platform. Since the initial report by Chen and Pleskow showing the feasibility of the Spyglass procedure in 35 patients,[14] a large number of studies, both retrospective and

Table 5 Conformity between patient management anticipated after single-operator cholangioscopy (SOC) and actually observed management

Actual management consistent with SOC findings	Overall		Indeterminate biliary strictures		Primary sclerosing cholangitis	
	N	%	N	%	N	%
Yes	54	88.5	43	89.6	11	84.6
No	7	11.5	5	10.4	2	15.4
Total	61	100	48	100	13	100

prospective, but none randomised, have been published. In 2015, two systematic reviews and meta-analyses were published, with slightly different methodologies and criteria for study inclusion: the review by Navaneethan *et al* including 10 retrospective and prospective studies (456 patients) aimed to determine the value of cholangioscopy-guided biopsies, whereas that by Xi Sun including 8 studies (335 patients), aimed at differentiating the value of visual impression from that of guided-biopsies.[15 16] In the first meta-analysis, a pooled sensitivity of 60.1% (95% CI 54.9% to 65.2%) was found for the diagnosis of malignant strictures against 69% (95% CI 57% to 79%) in the second one, whereas specificity of Spybite biopsy was 98% in both studies. However, Xi Sun *et al* calculated that visual impression had a higher sensitivity for malignancy at 90% (95% CI 73% to 97%), with specificity at 87%, but low positive and negative likelihood ratios at 7.1 (95% CI 3.8 to 13.3) and 0.12 (95% CI 0.04 to 0.33), respectively.[15 16] It is noteworthy that only four out of the studies on which both meta-analyses were based included patients with inconclusive ERCP and brushing,[17–20] meaning that many of the strictures explored in the other studies were 'indeterminate' insofar as CT and MRI were inconclusive, but possibly EUS-FNA and/or ERCP and brushings might have provided the information needed. Naveenathan *et al* calculated a sensitivity of SOC in those four studies of 74.7% (95% CI 63.3% to 84.0%), a specificity of 93.3% (95% CI 85.1% to 97.8%) and a pooled diagnostic odds ratio (DOR) of 46.0 (95% CI 15.4 to 138.1).[15] Perhaps even more interestingly, Siddiqui *et al*, in a retrospective analysis of 30 patients with eventually proven cholangiocarcinoma but negative ERCP and EUS-FNA findings, found that SOC had an accuracy of 77% for the diagnosis of malignancy.[19] In our study, prospectively including patients from nine referral centres, enrolment was limited to patients with strictly defined criteria, in whom no mass was easily amenable to EUS-FNA, no history suggested a definite diagnosis and at least one ERCP + brushing and/or fluoroscopy-guided biopsies were inconclusive. As in the above-mentioned reviews, we found sensitivity for malignancy to be moderate, with marginal differences between investigators and independent experts classifications of true and false cases. However, as also previously observed, visual impression provided slightly better sensitivity figures, both overall and in the IDBS group, but it is widely accepted that tumour boards do not decide oncological therapy on such subjective information. On the contrary, a visual impression of normality or mild inflammation tends to suggest upholding conservative management. Moreover, a visual impression of malignancy should not be overstated because, as shown by Sethi *et al*,[21] interobserver agreement on cholangioscopic features is at best slight in the absence of standardised, consensus-based terminology. We did not consider spyglass biopsies to be mandatory when visual impression (VI) was obviously benign since biopsies have a low negative predictive value and likelihood ratio. However, because VI remains subjective and there is no currently available consensual classification of SOC visual features, SOC-guided biopsies of any abnormal finding should therefore remain the rule. The sensitivity figures in patients with IDBS, relatively lower than in some previous studies, can be explained by the lower prevalence of malignant lesions, with two out of three patients eventually found to have a benign condition. This lower prevalence is also the consequence of strict criteria for enrolment, with all patients having a mass undergoing EUS-FNA and excluded from the study and some of the study patients having undergone more than one inconclusive ERCP. The study by Nguyen *et al* in 2013 showing that EUS-FNA can avoid the need for SOC in 60% of cases with difficult bile duct strictures strongly supports this approach.[22] Another possible contributor to decreased SOC diagnostic performance results from the methodology of the study, in which nearly all patients had a stent in situ at the time of enrolment, with the associated and potentially confounding inflammatory changes. However, this is also the 'real-life' situation, in which patients are often referred for SOC after one or several ERCPs in which prophylactic stenting is recommended.

Despite the moderate sensitivity of SOC for malignancy, our results, as analysed by investigators and independent experts, show that SOC has a major influence on patient management, an observation confirmed both overall and in both study subgroups. The benign findings in a majority of the patients have led to downgrade the initial diagnosis and preclude surgery in more than half of patients overall. Importantly, follow-up showed that false negative SOCs did not impair outcomes because further findings (including repeat SOC and brushings) redressed diagnosis before disease progression (see additional material). However, the potential to delay a diagnosis of cancer is clearly a limitation to keep in mind when discussing post-SOC indeterminate cases.

As important as changes in management suggested by SOC results in a given patient can be, they are irrelevant if these suggestions are not followed by consistent treatment adaptation, for example, if the MDT proposal sticks to an initially planned resection although SOC suggests a benign lesion with no need for surgery. The 11% rate of inconsistencies between patient management anticipated after SOC and the actually observed management can be deemed acceptable for a relatively new technique whose results may not be regarded as beyond any question.

We acknowledge that our study failed to reach the targets assigned to the PSC group, thus weakening the findings and precluding accurate measurement of diagnostic performance criteria for this subgroup. However, with regard to our main study endpoint, this underpowered group did not prevent finding a significant difference in the adequacy of suggested management to the definitive diagnosis between pre- and post-SOC assessment. In the PSC group, although SOC remained inconclusive in nearly one-third of patients, the proportion of inadequate managements was reduced by 63%, from 84.6% to 30.8%, and planned management was modified in more than two out of three patients. In the IDBS group, the inadequacy rate fell by 73%, and in the whole casemix by 71%.

Starting from these findings, two important issues should be raised: first, could the technique of SOC be improved in order to further reduce the number of inadequacies and more confidently guide professionals in their decision-making? Second, could the diagnostic strategy of biliopancreatic conditions and particularly IDBS, and the place of SOC in that strategy be modified to further improve outcomes? To address the first issue, the recently launched digital SOC system (Spyglass-DS) is certainly one significant step forward, with encouraging results from recent retrospective and prospective series.[23 24] Most recent cases of SOC performed in some of our centres with this new device were not included in our study, which may not fully reflect the current possibilities of this evolving technology, but it was methodologically necessary to base our assessment of SOC on the same technology for all the patients included. Even if this new generation brings more comfort of use and image reliability than fundamentally new technology, the only fact of feeling comfortable with taking a—still to be defined—sufficiently large number of biopsies and not be bothered by poor image quality and broken optic fibre is yet a significant progress. Better biopsies and easier targeting are certainly key factors in improving SOC diagnostic yield. The quality of Spybite biopsies has been compared with biopsies obtained with paediatric forceps with controversial results.[25 26] However, although cholangioscopic biopsies are necessarily small, one challenge will be to achieve deeper biopsy sampling in order to collect tumour cells buried within the thick fibrous stroma of most cholangiocarcinomas.[27] The second issue requires a better understanding of the optimal timing for SOC. In particular, it must be determined whether SOC must be contemplated after a first inconclusive ERCP with brushing, as is most commonly done currently, or should we rather perform SOC at the same time as a first ERCP, when other imaging modalities, especially EUS-FNA, remained inconclusive. The latter option would save a potentially precious time in the case of an invasive carcinoma and possibly allow for more clearcut distinction of malignant and inflammatory changes in bile ducts unaltered by weeks of stenting, but could also induce additional undue costs and morbidity. A valid answer to this question will require a randomised trial.

In conclusion, this study shows that single-operator and single-use peroral cholangiopancreatoscopy unveils a new era in the exploration of the most difficult biliopancreatic conditions, particularly IDBS, with a dramatic and positive impact on disease management. However, much remains to be done to improve the diagnostic performance of direct visualisation of intraductal diseases, with the implementation of a standardised semiology and specific terminology to describe anomalies, as well as a clarification of the optimal timing of SOC in the diagnostic workup.

Significance of this study

What is already known on this topic

- ERCP guided cytology/biopsy has low diagnostic yield.
- Biliary strictures without a mass and primary sclerosing cholangitis-dominant strictures are difficult diagnoses.
- Cholangioscopy can visualise lesions and target biopsies.
- Actual impact of cholangioscopy on patient outcome is unknown.

What this study adds

- Cholangioscopy induces changes in management in the majority of patients and avoids unnecessary surgeries.
- Although sensitivity for malignancy is moderate, cholangioscopy provides information necessary to optimise management in most patients with indeterminate biliary strictures.

How might it impact on clinical practice in the foreseeable future

- Cholangioscopy might be implemented in the workup of selected patients with challenging diagnosis, when a significant impact on outcome (essentially resection versus conservative management) is to be expected.

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Acknowledgements The authors wish to acknowledge the Clinical Research Unit (URC-Cochin) and its staff for their contribution throughout the study, as well as Prof Benoit Terris (BT), head of the Dept of pathology at Cochin hospital, Paris, for his expertise in most difficult cases.

Contributors FP designed and planned the study, contributed patients and data analysis, wrote and submitted the study. SL contributed patients and analysed data. FF was the methodologist and statistician for the study. TP, RéL, PB, FééM, DC, AC, BV and IB contributed patients. SC formalised the idea of a multi-centre prospective study of SOC. NK monitored patient inclusion and study quality. AL and SG played the role of external independent reviewers with regard to patient outcome classification.

Funding The study was funded through a grant from the National Cancer Institute (INCA).

Competing interests FP, RL and TP have received consultancy fees from Boston Scientific before, during or after the study.

Patient consent Obtained.

Ethics approval IRB approval from CPP Ile de France-III under N°Am5042-2-S.C.2564 and registered under EUDRACT N°2008-A00672-53.

Provenance and peer review Not commissioned; externally peer reviewed.

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6 Publication 4 [77]

Temporary placement of fully covered self-expandable metal stents for the treatment of benign biliary strictures

Ulriikka Chaput, Ariane Vienne, Etienne Audureau, Paul Bauret, **Philippe Bichard**, Dimitri Coumaros, Bertrand Napoléon, Thierry Ponchon, Jean-Christophe Duchmann, René Laugier, Hervé Lamouliatte, Bruno Védrenne, Marianne Gaudric, Stanislas Chaussade, Françoise Robin, Sarah Leblanc and Frédéric Prat

United European Gastroenterology Journal 2016, Vol. 4(3) 403–412

In a prospective study performed at the initial phase of use of partially covered self-expandable metallic stent (PCSEMS) placed with ERCP to treat benign stenosis, we validated the extirpability of PCSEMS for the treatment of anastomotic stenosis secondary to liver orthotopic transplantation 78. After gaining experience in this this procedure, we included in the present prospective study 92 patients in 10 tertiary centers to evaluate the use of fully covered self-expandable metallic stent (FCSEMS) placed with ERCP for 6 months for the treatment of benign biliary stenosis secondary to chronic pancreatitis (n = 42), liver transplantation (n = 36) or laparoscopic cholecystectomy (n = 14)

Results

- Stenting was successful in 100%
- Minor complications occurred in 25%
- Stent extraction was successful in 98%
- **Final success was obtained in 78% with a 12 months follow-up**

Impact for ERCP practice

FCSEMS removal after 6 months of implantation is generally easy. FCSEMS placement with ERCP is feasible and efficient for patients with biliary benign stenosis in nearly 80% of patients.

UNITED EUROPEAN GASTROENTEROLOGY
ueg journal

Original Article

United European Gastroenterology Journal
2016, Vol. 4(3) 403–412

DOI: 10.1177/2050640615606550
ueg.sagepub.com
SAGE

Temporary placement of fully covered self-expandable metal stents for the treatment of benign biliary strictures

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Abstract

Background: Endoscopic treatment of benign biliary strictures (BBS) can be challenging.
Objective: To evaluate the efficacy of fully covered self-expandable metal stents (FCSEMS) in BBS.
Methods: Ninety-two consecutive patients with BBS (chronic pancreatitis ($n = 42$), anastomotic after liver transplantation ($n = 36$), and post biliary surgical procedure ($n = 14$)) were included. FCSEMS were placed across strictures for 6 months before endoscopic extraction. Early success rate was defined as the absence of biliary stricture or as a minimal residual anomaly on post-stent removal endoscopic retrograde cholangiopancreatography (ERCP). Secondary outcomes were the final success and stricture recurrence rates as well as procedure-related morbidity.
Results: Stenting was successful in all patients. Stenting associated complications were minor and occurred in 22 (23.9%) patients. Migration occurred in 23 (25%) patients. Stent extraction was successful in all but two patients with proximal stent migration. ERCP after the 6 months stenting showed an early success in 84.9% patients (chronic pancreatitis patients: 94.7%, liver transplant: 87.9%, post-surgical: 61.5%) ($p = 0.01$). Final success was observed in 57/73 (78.1%) patients with a median follow-up of 12 ± 3.56 months. Recurrence of biliary stricture occurred in 16/73 (21.9%) patients.
Conclusions: FCSEMS placement is efficient for patients with BBS, in particular for chronic pancreatitis patients. Stent extraction after 6 months indwelling, although generally feasible, may fail in a few cases.

Keywords

Benign biliary strictures, metal stents, chronic pancreatitis, liver transplantation, endoscopic retrograde cholangiopancreatography, FCSEMS

Received: 26 March 2015; accepted: 23 August 2015

Introduction

Benign biliary strictures (BBS) may result from various causes, the most common being intra-operative biliary injury during cholecystectomy, inflammation and fibrosis in the course of chronic pancreatitis, and anastomotic strictures mainly due to ischemia and graft conservation impairment after liver transplantation. In the past decades, endoscopic retrograde cholangiopancreatography (ERCP) and stenting have been preferred as the first line treatment for BBS in consideration of their high initial success rate and morbidity

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lower than surgery. Until recently, the standard of care for the endoscopic management of BBS was using one or several plastic stents exchanged serially over a period of approximately one year.[1–3] Circa 2008, the advent of removable fully covered self-expandable metal stents (FCSEMS) opened a new paradigm for BBS management.[4] After initial studies demonstrated the feasibility of short temporary stenting with partially covered self-expandable stents,[5] and encouraging results were found in specific conditions such as post-liver transplant strictures,[6,7] outcomes of a modified strategy consisting in using only one, large bore FCSEMS for a shorter period of time with no intermediate exchange needed to be assessed in patients responding to the broader definition of common bile duct BBS. The present study was designed to evaluate prospectively the outcomes of the temporary placement of FCSEMS for benign biliary strictures in a multicenter setting.

Materials and methods

Patients

This study was conducted prospectively in ten French hospitals, under the aegis of the French Society of Digestive Endoscopy (SFED). Consecutive patients presenting with a BBS were considered for temporary placement of FCSEMS and included after informed consent was obtained. Inclusion criteria were a history of liver transplantation, chronic pancreatitis or biliary surgery, sustained impairment of liver function tests and a biliary stricture associated with ductal dilation detected by ultrasonography (US), computed tomography (CT), or magnetic resonance imaging (MRI), with a minimum distance of 2 cm between the upper stricture's edge and the lower limit of the main biliary confluence or liver hilum. Exclusion criteria were an uncertainty about the benign nature of the stricture, an intra-hepatic cholangitis and/or a stricture extending beyond the hilum, and previous interventions using metal stents. The study received IRB approval by the Comité de Protection des Personnes Ile de France 3 and received clearance by the AFSSAPS (2006-A00197-44).

Endoscopic protocol

Briefly, all procedures were performed with a large-channel duodenoscope under deep sedation using propofol. After selective biliary cannulation, a cholangiogram was obtained to evaluate the location and the severity of the stricture. Permalume®-fully covered Wallflex® stents (Boston Scientific, 10 mm in diameter, 40, 60, or 80 mm in length) were used in all patients. The choice of stent length was adjusted to the anatomy of the common bile duct and the length and level of the stricture. The proximal stent end had to be placed below the main hepatic confluence, in order to prevent occlusion of one biliary branch by the stent cover. Performing a biliary sphincterotomy was not mandatory, but was usually done to allow for a full expansion of the stent through the papilla, make extraction easier and prevent pancreatitis due to pancreatic duct occlusion by the stent cover. Stricture dilation with a balloon before placing the stent was not recommended systematically but was allowed, especially in case of a tight stricture with difficult passage of a catheter.

FCSEMS were left in place for 6 months and then removed using a snare and/or a rat tooth forceps, by grasping the metallic loop at the distal end of the stent. As to assess biliary patency, a cholangiogram was performed immediately after stent removal, by using a 12 mm retrieval balloon passed downwards through the stricture with contrast liquid being injected above the balloon. The bile duct was deemed patent with satisfactory sizing when the fully inflated balloon was passed without any significant resistance and if there was no evidence of contrast retention above the initial stricture site. Antibiotics were systematically administered prophylactically before each ERCP.

Follow-up

Patients were followed through a combination of clinical examination and liver function tests at 1, 3, 6, 9, and 12 months after FCSEMS removal. In case of recurrent stricture, follow-up was stopped and the patient was offered a new treatment whose choice was left to the investigator's decision.

Statistical analysis

Descriptive results are reported as means (± standard deviation (SD)) or medians (range and inter-quartile range) for continuous variables, and as count (percentage) for categorical data. Univariate analysis of the patient or procedures' characteristics associated with outcomes of interest (i.e. initial success, migration, recurrence) were performed using Chi^2 tests or Fisher's exact test for categorical variables, and unpaired t-tests or Wilcoxon rank-sum tests for continuous variables to account for non-normality of the variable distributions when demonstrated by the use of the Shapiro–Wilk test for normality. Multivariate analysis was conducted for potential predictors associated at the $p < 0.2$ level in univariate analysis using Firth's bias correction to account for the small sample size. A stepwise approach was followed by removing non-significant variables at each step. Censored data (time to recurrence as the endpoint) were analyzed using univariate survival analysis by the log-rank test and multivariate Cox proportional hazards models fitted to assess the independent role of each predictor. A *p*-value < 0.05 was considered statistically significant. All statistical analyses were performed using Stata version 12.1 (StataCorp, College Station, TX, USA). Based on previously reported results and our own experience, a sample size of at least 80 subjects was required to estimate the primary endpoint with an accuracy of ± 10%, assuming an expected early success rate of at least 70%.

Results

Ninety-two patients (68 men, 24 women) aged 54.4 ± 9.9 years (median 55; range 15–78) were included in ten centers (a mean of 9.2 ± 12 patients per center). The etiology of the biliary stricture was chronic pancreatitis ($n = 42$), anastomotic after liver transplantation ($n = 36$) and post-surgical biliary procedure, mainly cholecystectomy ($n = 14$). Forty-five (48.9%) patients had at least one previous endoscopic treatment of the same stricture with dilation and/or plastic stenting. Table 1 summarizes endoscopic treatments prior to inclusion in the study.

FCSEMS placement was performed successfully in all patients. Twenty-six (28.3%) patients underwent an endoscopic balloon dilation before stenting, at a median diameter of 8 mm (mean = 7.44 ± 0.92 mm, range 6–8).

Icterus resolved in all jaundiced patients after stenting, and liver function tests improved in all other patients. Complications occurring within five days of stenting were observed in 11 (11.9%) patients, all of which were minor. Five patients, all of whom had undergone a standard ($n = 3$) or precut ($n = 2$) sphincterotomy developed a minor post-ERCP pancreatitis. One patient presented a minor hemorrhage treated endoscopically per procedure by a local injection of epinephrine. Four patients had transient abdominal pain without hyperlipasemia and one developed a cholecystitis which promptly responded to antibiotics and did not require surgery.

Table 1. Endoscopic treatments prior to inclusion in the study

No previous treatment	47 (51.1%)
Previous treatment	45 (48.9%)
Plastic stent alone	33 (35.8%)
Balloon dilation alone	2 (2.1%)
Balloon dilation associated with plastic stent	16 (17.4%)
Mean number of previous ERCP (SD); median (range)	0.8 (± 1.13); 0 (0–6)

Delayed complications (between five days and 6 months) occurred in 11 (11.9%) patients. Hepatobiliary infection occurred in the form of cholangitis in six patients and liver abscess in one. Biological abnormalities (cholestasis and/or jaundice) without any clinical sign occurred in four other patients.

Stent migration occurred in 23 (25%) patients. The stent migrated distally in eighteen patients: either completely (the stent was no more attached to the papilla) in twelve patients, or partially (stent dislodged below the stricture but still attached to the papilla) in six patients. Proximal migration (stent dislodged above the stricture) occurred in five cases. Twelve out of 23 patients (52.2%) with stent migration presented a persistent stricture requiring further endoscopic treatment. Most persistent strictures were post orthotopic liver transplantation (OLT) ($n = 5$) or post cholecystectomy ($n = 6$), only one stricture was due to chronic pancreatitis. These clinically significant stent migrations were observed a mean of 20.8 ± 8 weeks after stent implantation. Nine of 23 (39.1%) patients with stent migration had undergone balloon dilation at the time of stenting.

Stent removal was possible in all but two patients with proximal migration of the stent. One of them presented with a severe chronic pancreatitis and developed a duodenal obstruction secondary to cystic dystrophy, in which the stent is unlikely to have any causal relationship. Duodenal obstruction made it impossible to remove the stent endoscopically, and the patient subsequently underwent a Whipple's procedure, during which the stent was removed. The other one died later from cardiovascular disease, with no biliary symptom or complication. Removal was found to be demanding in two types of situation: impaction of the distal end of the stent in the papilla ($n = 2$) and proximal migration ($n = 2$) of the stent. Argon Plasma destruction of the hyperplastic tissue covering the distal end of the stent was required in two patients to successfully achieve stent extraction.

Early success of endoscopic treatment, was 84.9% per-protocol ($n = 73$), but 79.3% in an intention-to-treat (ITT) perspective. Success rates relative to the etiology of BBS were 87.9%, 94.7%, and 61.5% in patients with BBS due to liver transplantation, chronic pancreatitis, and post-surgical, respectively. ERCP after the 6 months sizing period showed a persistent stricture in 11 (11.9%) patients, ten of whom had had a partial ($n = 3$) or a complete ($n = 7$) distal migration of the metal stent. Table 2 displays outcomes after the stenting period.

Final success defined as a sustained biliary stricture resolution was observed in 57/73 (78.1%) patients (61.9% from an ITT perspective) with a median follow-up of 12 (± 3.56 SD) months**.** Recurrence of biliary stricture after early success occurred in 16/73

(21.9%) patients. All recurrences developed at the original site of the stenosis. In particular, there was no evidence of a new stenosis at the proximal edge of the original FCSEMS. The mean time to recurrence of biliary stricture was 4.2 (±3.3 SD, range 0.5–12) months. Table 3 displays the outcomes at the end of follow-up. One patient died of pulmonary embolism before any treatment; data from two cases were unavailable. Overall outcomes are summarized in Figure 1.

Univariate statistical analysis showed that stent migration was associated with female sex ($p = 0.013$), post-transplant and other post-surgical BBS ($p = 0.006$), and a higher number of endoscopic procedures before FCSEMS placement ($p = 0.026$), such as endoscopic dilations ($p = 0.025$). However, endoscopic dilation at the time of FCSEMS placement was not associated with more stent migrations (see Supplementary table 1 online). In the multivariate analysis of risk factors for stent migration, the etiology of the stricture was independently predictive, chronic pancreatitis being a protective factor (Table 4).

Table 2. Outcomes and management at the end of the 6 months stenting period

ERCP findings	n (%) patients	Management
Failed stent extraction	2 (2.3%)	Surgery, $n = 1$, conservative, $n = 1$
Persistent stricture	11 (13.25%)	Dilation, $n = 1$
		Dilation + plastic stent, $n = 1$
		Dilation + 2nd FCSEMS, $n = 3$
		Plastic stent, $n = 1$
		2nd FCSEMS, $n = 5$
Minimal residual anomaly	46 (53.5%)	Observation
No stricture	27 (31.4%)	Observation
Early success	73 (84.9%)	Observation

Risk factors for the persistence of a stricture in the univariate analysis were found to be female sex ($p = 0.04$), stent migration ($p < 10^{-4}$), and the etiology of the stricture ($p = 0.01$). A significantly better stricture resolution rate was found in patients with chronic pancreatitis (94.7%) than in those with liver transplant (87.9%) and post-surgical (61.5%) ($p = 0.01$) strictures. However, the difference between chronic pancreatitis patients and transplanted patients did not reach significance ($p = 0.33$, odds ratio (OR) = 2.23, confidence interval (CI) 0.44–11.3) probably due to the small sample size (See Supplementary table 2 online). In a multivariate analysis, etiology was the only significant variable associated with early success with better results for chronic pancreatitis patients compared to post-surgical patients and liver transplant patients ($p = 0.021$, OR = 15.5, CI 1.5–160.4) (Table 5).

Finally, stricture recurrence was associated in univariate analysis with dilation before stent placement ($p = 0.009$, hazard ratio (HR) 3.68, CI 1.37–9.91) and stricture etiology. Higher recurrence rates were found in post-surgical ($p = 0.007$, HR 7.87, CI 1.76–35.28) and post-transplant strictures ($p = 0.04$, HR 3.99, CI 1.08–14.73) when compared to chronic pancreatitis strictures. No variable was found to be independently associated with stricture recurrence in the multivariate analysis (Table 6).

Discussion

ERCP has recently become the first line treatment of benign biliary strictures as a reward of a better safety

Table 3. Outcomes at the end of the follow-up (1 year after stent extraction)

ERCP findings	n (%)	Management	n
Stricture recurrence	16/73 (21.9%)	Dilation + plastic stent	3
		2nd FCSEMS alone	3
		Dilation + 2nd FCSEMS	4
		Surgical treatment	3
		Unavailable data	2
		Death from other cause	1
Final success (sustained stricture resolution):		Observation	
- Per-protocol	57/73 (78.1%)		
- ITT	57/92 (61.9%)		

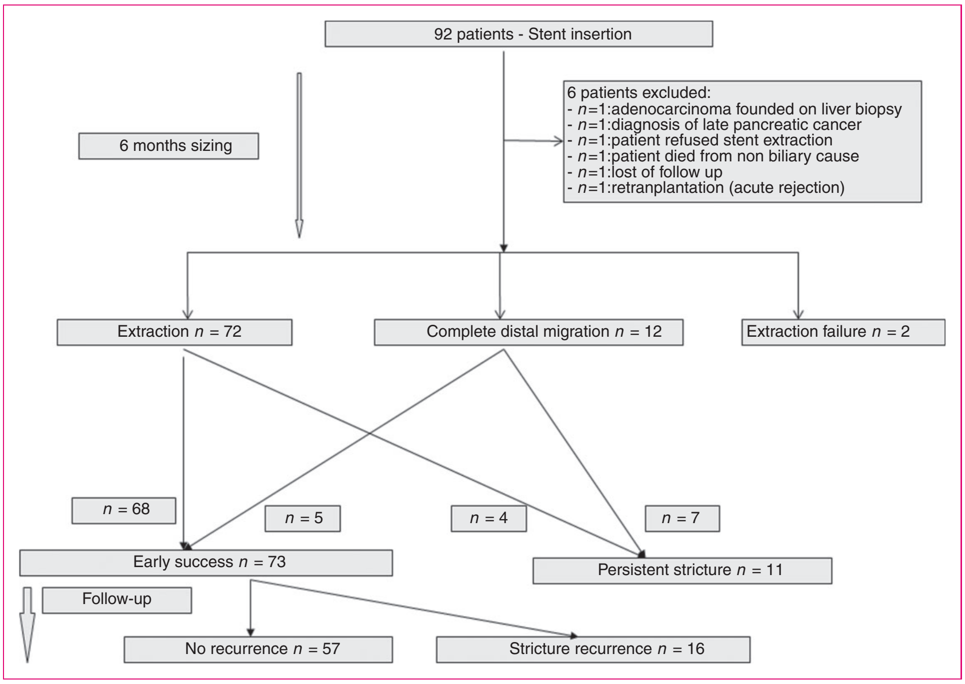

Figure 1. Flow chart summarizing overall outcomes.

Table 4. Multivariate analysis of patient's and procedural's characteristics associated with stent migration

		p-value	95% CI
Age		NS	
Sex	Male	NS	
	Female		
Etiology	Liver transplantation	**0.009**	1.5–16.34
	Chronic pancreatitis	1 *reference*	
	Post-surgical	**0.009**	1.62–28.77
No. of previous endoscopic treatments		NS	
Dilation in a previous procedure	No	NS	
	Yes		
Dilatation preceding FCSEMS placement	No	NS	
	Yes		
Dilation diameter	6 mm	NS	
	8 mm		
Stricture length		NS	
FCSEMS length	40 × 10 mm	NS	
	60 × 10 mm		
	80 × 10 mm		

Table 5. Multivariate analysis of patients' or procedure's characteristics associated with early success

		p-value	95% CI
Age		NS	
Sex	Male	NS	
	Female		
Etiology	Liver transplantation	0.835	0.023–20.7
	Chronic pancreatitis	1 *reference*	
	Post-surgical	**0.021**	1.5–160.45
Number of previous endoscopic treatments		NS	
Dilation in a previous procedure	No	NS	
	Yes		
Dilatation preceding FCSEMS placement	No	NS	
	Yes		
Dilation diameter (mm)	6	NS	
	8		
Stricture length		NS	
FCSEMS length	40 × 10	NS	
	60 × 10		
	80 × 10		
Migration	No	NS	
	Yes		

Table 6. Univariate analysis of patient's or procedure's characteristics associated with final success

Variable		*n*	p-value	Hazard ratio IC95%		
Age		69	0.753	0.992	0.943	1.043
Sex	Male	51		1 *reference*		
	Female	18	0.5735	1.35	0.47	3.9
Etiology	Liver transplantation	28	**0.0381**	3.99	1.08	14.73
	Chronic pancreatitis	33		1 *reference*		
	Post-surgical	8	**0.007**	7.87	1.76	35.28
Number of previous endoscopic treatments		69	0.1451	1.425	0.885	2.295
Dilation in a previous procedure	No	50		1 *reference*		
	Yes	11	0.8622	1.12	0.32	3.96
Dilation preceding FCSEMS	No	45		1 *reference*		
	Yes	20	**0.0098**	3.68	1.37	9.91
Dilation diameter (mm)	6	5		1 *reference*		
	8	10	0.1778	0.38	0.09	1.55
Stricture length		61	0.1521	0.614	0.315	1.197
Stent length	40 × 10	19		1 *reference*		
	60 × 10	17	0.1718	4.61	0.52	41.24
	80 × 10	28	**0.0385**	8.69	1.12	67.37
Migration	No	59		1 *reference*		
	Yes	10	0.5945	1.41	0.4	4.94
Persistent stricture	Attenuated	43	0.8855	1.08	0.39	2.97
	No	26		1 *reference*		

profile than interventional radiology and surgery, but its best modalities are still debated. The simultaneous and temporary placement of multiple plastic stents is the current standard, as recently recommended by ESGE. This modality showed good long term results, usually over 70%, except in studies including patients with chronic pancreatitis which reported long term success of 31–62%.[8,9] However, this approach, requiring frequent endoscopic procedures, usually every 3 months during one year, can be impaired by poor compliance and intercurrent side effects, thus increasing morbidity and raising costs. Fully covered metal stents were developed in the 2000s initially for the prevention of tumor growth during palliative stenting. Unlike uncovered stents used for malignant conditions, FCSEMS can be extracted and therefore placed for a limited period of time in benign strictures. Recent data from prospective studies have been encouraging, with stricture resolution rates at stent removal of 43-90%, although recurrences occurred in 6–47%.[4,7,10–15] A summary of major results from those studies is displayed in Table 7.

Our study included a large number of patients from both academic and community hospitals as well as private clinics, thus representative of the full range of BBS patients, all treated along a standardized endoscopic protocol and using only one type of FCSEMS. Some predictable drop-outs were due to initially unsuspected diagnoses of malignancy and the unexpected need for surgery during follow-up, but only one patient was lost at follow-up during the stenting period while less than 10% were lost during follow-up after stent removal. The main study limitation was the non-randomized design, making comparisons with conventional treatment outcomes (i.e. plastic stenting) speculative.

In this study, the mean time to recurrence of biliary stricture was relatively short (4.2 ± 3.3 SD, range 0.5–12 months). However, the early success (85%) and long term success (78.1%) rates observed in our study are similar to those previously reported with FCSEMS and compare favorably with those of plastic stenting.[16] Moreover, most recurrences occur within the first year of plastic stent removal, which is consistent with our findings. The main hindrance to the efficacy of FCSEMS in BBS appeared to be stent migration, observed in nearly 25% of patients, with a particular burden from distal migrations. It is noteworthy that nearly all (10 out of 11) strictures persistent at the end of the stenting were in patients with stent migration, although only 55% of migrations led to a persistent stricture, thus suggesting that the stent can remodel the bile duct in certain cases even when it does not stay in place as long as expected. Migration was more frequent in patients with short strictures due to liver transplantation or after cholecystectomy than in patients with BBS during chronic pancreatitis, thus possibly explaining in part the better results in chronic pancreatitis patients. In liver transplant and also some post-surgery BBS, migration is probably more likely when the stricture is at the same time short and close to the liver hilum, leading to an imbalance in the length of stent above and below the stricture. Multiple plastic stents or FCSEMS equipped with specific anti-migration features could be preferred for such cases, were this observation be confirmed by further studies. Migration could also be favored by dilation at the time of stenting, but our findings do not support this hypothesis, since only dilations performed during previous endoscopic therapies were associated with a higher migration rate, whereas only 39% of patients with stent migration had undergone balloon dilation at the time of stenting. Whether or not to dilate the stricture before implanting an FCSEMS remains therefore open to the endoscopist's choice, with dilation to be considered when the stricture is very tight or its location too close to the hilum to allow for expansion and self-shortening without stent dislocation. Park *et al.* reported a series of 22 patients treated with metal stents with anti-migration flaps.[11] No case of migration was observed in this series, but the rate of post-stenting pancreatitis was unusually high at 18%. Flared-end stents did not appear to reduce the migration rate up to 30–40% in two studies.[11,12] Of several other solutions proposed in order to reduce the migration rate of FCSEMS (placing the stent without prior sphincterotomy, using systematically long FCSEMS or stitching the stent to the duodenal mucosa by using clips), none has been validated by prospective studies.

It is noteworthy that nearly 50% of patients in this study had a refractory stricture, defined as a stricture which had not responded to one or several previous endoscopic treatment. This study shows that metal stents can be used as a second line treatment and still present a good efficacy profile. An important finding is that chronic pancreatitis patients (45.6% of the patients included), whose strictures are deemed difficult to treat with plastic stents, had favorable outcomes and may therefore benefit from this type of stent, not least because they might be easier to convince of this expeditious, two-step treatment. On the contrary, it was bewildering to find transplantation and biliary surgery-associated strictures bearing a significantly poorer prognosis in terms of long term success rate than chronic pancreatitis strictures, although second-line stentings were not significantly less common in the latter subgroup (43% vs 48%). However, despite several reports of lower stricture resolution rates among chronic pancreatitis patients,[4,12] one recent international study found a 79.7% stricture resolution rate after 10-12 months of FCSEMS stenting in this

Table 7. Peer-reviewed reported results concerning fully-covered self-expandable metal stents for benign biliary strictures

Study	*n*	Stent design	Time to stent removal median (months)	Successful stent removal	Stricture resolution at stent removal	Migration rate	Morbidity	Median follow-up (months)	Recurrence rate
Mahajan et al.[4]	44	Fully covered	3.3	93%	83% (77% ITT)	4.8%	24.4%	-	-
Traina et al.[7]	16	Fully covered	2	100%	87.5%	37.5%	0%	10	6.25%
Park et al.[11]	43	22 with flaps	6	100%	91%	0%	31.8%	4	14%
		21 flared ends		100%	88% (71.4% ITT)	33%	23.8%		19%
Tarantino et al.[14]	54	Fully covered	2	100%	Group 1 71.8%	33%	0%	22.1	14.3%
100%					Group 2 53.3%	46.7%	0%	14.4	25%
Perri et al.[12]	17	7 Standard FC	6	100%	43%	100%	57%	24	57%
		10 Flared ends		100%	90%	40%	10%		30%
Tarantino et al.[13]	62	Fully covered	3	98.4%	90.3%	24.2%	1.6%	15.9	7.1%
Kahaleh et al.[15]	133	Fully covered flared ends	3	72.9%	73.8%	10.5%	11.3%	1	-
Devière et al.[10]	187	Fully covered	4–6* 10–12**	100%	76.3%	31.1% (complete: 9%)	27.3%	20.3	14.8%
Current study	**92**	**Fully covered**	**6**	**97.8%**	**85% (79% ITT)**	**25% (Complete: 13%)**	**23.9%**	**12**	**21.9%**

ITT: intention-to-treat.
Time to stent removal in this study was:
*4–6 months in the liver transplant group;
**10–12 months for chronic pancreatitis and cholecystectomy patients.

subset of patients,[10] who could thus benefit from an endoscopic treatment with a metal stent and only two procedures. The main reason for poorer outcomes in OLT patients, in contrast to other studies in which somehow better results were reported with FCSEMS,[7,13] can be related to the particular nature of OLT strictures and also to a higher stent migration rate in this subgroup. A different composition of anastomotic strictures in fibroblasts, inflammatory cells and elastic fibers may account for a higher elasticity and resilience of those strictures, when compared to post-cholecystectomy and chronic pancreatitis ones. However, pathologic studies are lacking to support this hypothesis. Another hypothesis may result from the observation that most anastomoses were performed pretty close to the hilum, with only a short portion of the graft's common bile duct available for stent implantation above the stricture and below the main confluence, an anatomic feature which makes stents prone to migration.

Finally, stenting duration might crucially influence outcomes. Stent migration was found to negatively affect outcome primarily by reducing the duration of stenting. One could also think that a protracted stenting period would yield better results, as suggested by the experience of plastic stents, yielding better outcomes after 1 year than after shorter periods. We chose a period of 6 months because we had enough experience with the stents used in the study to have little doubt as to their removability after this timespan, whereas 2 or 3 months was more common in previous studies.[4,6,7,13,14] We were also aware that a serious matter for concern is removability of stents after a long indwelling, if the stent cover is too damaged to prevent impaction in hyperplasia and fibrotic-inflammatory tissue, a problem commonly encountered with partially covered stents. In the above quoted study by Devière et al.,[10] after a stenting period of 10–12 months, 4% of scheduled stent removals needed multiple attempts due to difficult extraction and 14% required an anticipated removal due to complications. In that study the stricture resolution rate after stent removal was 76.3%, as against 79.3% in the present study, suggesting that a longer stenting is not necessarily associated with better outcomes.

In conclusion, FCSEMS placement is feasible and efficient for patients with BBS in nearly 4 out of 5 patients, including those with previous standard endoscopic treatments. FCSEMS removal after 6 months of implantation is generally easy, but there is a caveat on the uncommon event of a failure to remove an impacted stent. Best results seem to be obtained in chronic pancreatitis patients, whereas outcomes in other types of strictures (post-OLT and cholecystectomy) may be hampered by more frequent stent migration. Better initial and long term results could be expected if stent migration rates were lower, a goal that improving stents' design is more likely to achieve than modifying endoscopic protocols.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest

None declared

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7 Publication 5 [73]

A European survey of perendoscopic treatment of biliary complications in patients with alveolar echinococcosis

Sylvain Ambregna, Stéphane Koch, Michael C. Sulz, Beate Grüner, Sümeyra Öztürk, Jean-Baptiste Chevaux, Małgorzata Sulima, Andrea de Gottardi, Bertrand Napoléon, Armand Abergel, **Philippe Bichard**, Isabelle Boytchev, Pierre Deprez, Jerome Dumortier, Jean-Louis Frossard, Eric Kull, Bernard Meny, Darius Moradpour, Fréderic Prat, Geoffroy Vanbiervliet, Thierry Thevenot, Dominique Angèle Vuitton, Solange Bresson-Hadni & Lucine Vuitton

In this European multicenter study, 38 patients were included in 18 tertiary centers for treatment of biliary complications (26/38 biliary stenosis) of alveolar echinococcosis with ERCP placement of plastic or cSEMS.

Results

- Stenting was performed in 30/38 patients with a median of 3 ERCP
- 139 plastics stents were inserted during 85 ERCP
- 11 cSEMS were inserted during 11 ERCP
- Resolution of symptoms within 7 days was reported in 95% of cases
- **Prolonged biliary patency was obtained in 73% of uncomplicated patients with a median follow-up of 50 weeks**
- Cholangitis was reported in 10% of case and may be prevented by intensive biliary duct lavage.

Impact for ERCP practice

In patients with alveolar echinococcosis related bile duct stenosis, this study suggests than repeated per-endoscopic interventions is efficient to obtain long-term bile duct patency

EXPERT REVIEW OF ANTI-INFECTIVE THERAPY, 2017
VOL. 15, NO. 1, 79–88
http://dx.doi.org/10.1080/14787210.2017.1252260

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ORIGINAL RESEARCH

A European survey of perendoscopic treatment of biliary complications in patients with alveolar echinococcosis

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ABSTRACT
Background: Biliary complications represent a turning point in the course of Alveolar Echinococcosis (AE). We conducted a European survey to collect data on the current usage and results of perendoscopic interventions (PEIs) for their treatment.
Methods: Patient's characteristics and follow-up until January 31st, 2015 were recorded using an online questionnaire.
Results: From 18 centers 129 PEIs were analyzed in 38 patients; 139 plastic stents were inserted during 85 PEIs; median time between stent placements was significantly longer when 3 stents or more were placed. Initial symptoms disappeared in 95% and long-term bile duct patency was obtained in 73% of cases. Cholangitis was a more frequent complication of the PEIs (10%) than in other indications; intensive lavage of the bile ducts may prevent this complication.
Conclusion: European centers use perendoscopic biliary drainage as an efficient and safe alternative to surgery to treat AE biliary complications. Insertion of multiple plastic stents delays stent occlusion and leads to effective and prolonged bile duct patency.

ARTICLE HISTORY
Received 26 July 2016
Accepted 20 October 2016

KEYWORDS
Endoscopic retrograde cholangio-pancreatography (ERCP); alveolar echinococcosis (AE); Echinococcus multilocularis; biliary stricture; biliary stent; cholangitis

1. Introduction

Human alveolar echinococcosis (AE) is a rare parasitic disease caused by the development of the larval form (metacestode) of the cestode *Echinococcus multilocularis*. The endemic areas are located in Central and Eastern Europe, Russia, Central Asia, China, northern Japan, Alaska [1], as well as Canada and central-northern USA where *E. multilocularis* infection is emerging [2]. In Europe, in highly endemic areas, the annual incidence of AE is 2/100,000; there is an increase of incidence in previously identified endemic areas and AE is now present in countries previously considered free from the disease, such as Eastern-Central and Baltic States [3].

The parasite larva develops in the liver like a slowly growing cancer [4], surrounded by an intense granulomatous and fibrotic reaction. The biliary tree is often involved in the parasitic process and/or compressed by fibrosis. In advanced cases, necrosis occurs at the center of the parasitic lesion and leads to complications, dominated by bacterial infection or communication of the necrotic cavity with the bile ducts [5–7]. Radical surgery is the only effective treatment [8]. Liver transplantation may be an alternative in locally advanced stages when radical hepatectomy is not possible [9]. However, in 70% of cases, only palliative treatment can be proposed combining a parasitostatic treatment with benzimidazole (albendazole) and the treatment of the complications of the disease [8]. Nevertheless, nowadays the global prognosis of the disease has markedly improved [10].

Biliary complications (cholangitis, sclerosing cholangitis-like lesions, stenosis of the bile ducts, intrahepatic bile duct lithiasis, biliary fistulas, secondary biliary cirrhosis, and post-operative biliary stenosis after hepaticojejunostomy) are the main

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issue in AE management and account for the burden of the disease. They occur in at least one-third of AE patients who cannot be operated on radically, either at diagnosis or later despite prolonged albendazole treatment [11,12], and represent a 'turning point' in the course of AE [7,11]. The palliative treatment of these biliary complications is not standardized. Since the 1990s, clinicians in charge of AE patients have moved from surgical derivation toward an increasing use of radiology-guided and perendoscopic techniques [5,8,13,14]. In the absence of any controlled trial, and based on small retrospective series [11], the last expert consensus on AE diagnosis and treatment stated that surgery should be avoided whenever the lesions could not be removed radically [8]. Radiological percutaneous transhepatic biliary drainage (PTBD) is less invasive than palliative surgery, but often requires an external drain for several months and occasionally for life [5]. In addition to usual complications [14–16], the frequent communication between the necrotic parasitic lesion and the biliary tree causes frequent obstruction of the drain by migration of necrotic/parasitic plugs and requires regular drain removal and replacement [5].

Endoscopic drainage by endoscopic retrograde cholangiopancreatography (ERCP), designated below by 'perendoscopic intervention' (PEI), may be an alternative to PTBD and has the advantage of avoiding the discomfort of an external drain [14,17]. In cystic echinococcosis (CE, or 'hydatid disease,' due to *Echinococcus granulosus sensu lato*), PEIs showed good efficacy [18]. However, hepatic lesions in AE are different from those observed in CE. The cystic structures of CE compress the bile ducts whereas AE lesions invade them. After surgery, ERCP is used to treat biliary fistulas in CE, while irreversible postoperative bile duct stenosis is the major complication in AE [14]. Only a few case reports and single-center retrospective experience with little clinical and technical details on the PEIs were published for AE [11,12,19–21]. As AE is a rare disease, to alleviate the case recruitment issue we conducted a retrospective multicenter European survey which aimed at supporting international recommendations for the care management of AE patients with biliary complications in the future. We collected data on the current usage of PEIs in AE in Europe, their technical aspects, efficacy and safety, as well as on AE patient's outcome after PEIs.

2. Methods

2.1. Recruitment of patients

All European physicians working in university hospitals or regional hospitals in charge of patients with AE or practicing ERCP were recruited directly and/or through various professional and scientific associations to participate in the study.

The Ethical Committee of Besançon University Hospital approved this study on 15 February, 2012; this agreement and its rationale were submitted to local Ethical Committees of other centers. Physicians included their cases in the survey between 1 May 2013 and 31 January 2015. Inclusion criteria of patients in the database were as follows: diagnosis of AE confirmed by pathology or molecular identification of *E. multilocularis* or assessed by imaging evidence and a positive serological test (Western blot, specific of *Echinococcus multilocularis*) according to international recommendations [8]; PEI performed for AE biliary complication; and patients treated in European countries whatever the date of endoscopic treatment. Patients treated for CE were excluded from this survey. After inclusion of cases, patients with missing data for the main technical and/or outcome items were excluded from the analysis.

2.2. Online data recording

The physicians completed an online questionnaire via an internet-based tool (LimeSurvey software – https://www.limesurvey.org – adapted to the AE survey by *Emosist*®, Besançon, France) [22]. Data regarding the following domains were recorded: demographic patient's characteristics; AE history; staging according to the WHO-Informal Working Group on Echinococcosis PNM classification (P: parasitic lesions, N: invasion of neighbor organs, M: metastasis) [8,23]; previous treatments (including surgery, radiological drainage, chemotherapy); symptoms preceding endoscopic management; biological parameters; perendoscopic techniques (including 'intensive biliary lavage,' defined by the infusion of a minimum of 250 mL of saline in the bile ducts); associated treatments (including antibiotics); and patient's follow-up (until 31 January 2015).

2.3. Clinical end points

A successful PEI was defined by the resolution of the initial symptom(s) indicating ERCP within 7 days. If other therapies for biliary drainage were necessary in case of failure of PEI, the modality was documented. Early complications (within the first 30 days) and later complications of PEIs were recorded. Definition of stent obstruction was left to each investigator's judgment. 'Biliary patency' in patients with stenting procedures was defined by the definitive removal of the stent without recurrent biliary complication until the latest news. The time criterion to differentiate between 'early' and 'late' biliary complications of AE was before and after the end of the 3rd year after AE diagnosis respectively, as proposed by Frei et al. [11].

2.4. Statistical analysis

Descriptive statistics for clinical characteristics, per-endoscopic procedures, and patient outcomes were expressed as median (CI95%) or number (percentage). Comparisons of continuous variables between groups of patients were performed using nonparametric Mann–Whitney and Kruskal–Wallis tests. For comparisons of categorical variables, a Chi-square or the Fisher exact tests were used. P values less than 0.05 were considered significant. Statistical analysis was performed using *NCSS*® software.

3. Results

3.1. Recording centers and AE patients' characteristics

Figure 1 shows the location of the 20 centers which answered our request of participation. Among these centers, 14 AE referral

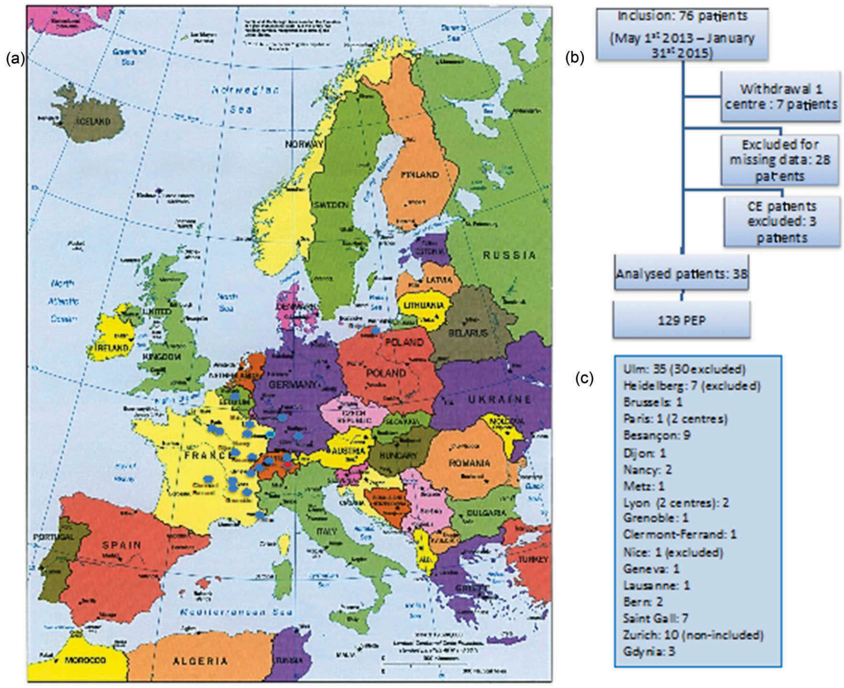

Figure 1. Map of the digestive endoscopy centers participating in the EndoscEchino survey (a); Flow chart of patients inclusion in the EndoscEchino survey (b); Patients treated with per-endoscopic procedures (PEI) for biliary complications of alveolar echinococcosis in each center (c).
CE: cystic echinococcosis.

centers were reached through direct contact and 6 digestive endoscopy centers contacted us after receiving information through professional or scientific associations. Patients from Zurich, Switzerland could not be included because of local ethical issues. The number of patients treated for AE biliary complications with ERCP ranged from 1 to 35 per center and the total number of PEIs for AE ranged from 1 to 112 per center. Twenty-eight patients were secondarily excluded because of numerous missing data, 7 patients recruited in 1 center because of secondary withdrawal from the study by local investigators, and 3 patients because biliary obstruction turned to be due to CE. A total of 38 patients (28 males and 10 females) in 18 centers underwent 129 PEIs and were included in the final analysis (Figure 1).

Patient median age was 52 years [7–83] at the time of diagnosis of AE and 56 years at the time of the first PEI. The 'P' value (parasite location) of the PNM classification at the time of diagnosis of AE was P4 in 17 (44%) patients. Common hepatic duct was involved in 19 (49%), bile duct bifurcation in 12 (31%), right hepatic duct in 3, and left hepatic duct in 2 patients; location was not specified in 2 patients (Table 1). Biliary complications were concomitant to AE diagnosis in 24 patients (61%). The first PEI was preceded by several episodes of cholangitis in 20 patients (51%).

3.2. Previous non-endoscopic treatments

Before the first PEI, 16 patients (41%) were not treated with benzimidazoles; all these patients were diagnosed during the month before the first PEI. Fifteen patients (38%) had previous surgical interventions for AE. The aim of surgery was: curative for 10 patients; palliative for 2 patients; diagnostic (explorative laparotomy) for 2 patients; and not specified for 1 patient. Eight patients had ultrasound-guided PTBD before the PEI (Table 1).

3.3. Perendoscopic interventions

One hundred twenty-nine PEIs were performed between 1997 and 2014. The first PEI in a patient with AE was performed in 1997 and was the only one before 2000; between time periods 2000–2004, 2005–2009, and 2010–2014: 11, 42, and 75 PEIs were performed, respectively. The first PEI was performed within the first 3 years after diagnosis in 30 patients (77%) (Table 1). Each patient had a median of 3 PEIs [1–11]. Indications for PEIs were jaundice or chronic anicteric cholestasis (32%), cholangitis (26%), biliary pain (20%), radiological drainage failure (5%), and surgical drainage failure or surgical

Table 1. Characteristics of the patients with alveolar echinococcosis (AE) included in the EndoscEchino European survey.

Patient	*Duration of AE before first PEI (years)*	*Patient age at first PEI (years)*	*P/N/M*	*Location of lesion on biliary tract (Bismuth classification)*	*Previous treatments*		
					Surgery (purpose)	*PTBD*	*BMZ drugs*
BE 1.1	3	76	4/0/0	Common hepatic duct (I)	Exploratory	_	Yes
FR 1.1	6	59	4/1/0	Common hepatic duct (I)	Curative, but recurrence	_	Yes
FR 1.2	15	49	3/*NA*/*NA*	Bile duct bifurcation (II)	Curative	_	Yes
FR 1.3	5	40	4/1/0	Bile duct bifurcation (II)	_	Yes	Yes
FR 1.4	24	84	3/1/*NA*	Bile duct bifurcation (II)	Palliative	_	Yes
FR 1.5	0	83	3/0/0	Bile duct bifurcation (II)	_	_	_
FR 1.6	0	75	3/0/0	Bile duct bifurcation (II)	_	_	_
FR 1.7	22	73	4/1/0	Common hepatic duct (I)	Palliative	_	Yes
FR 1.8	2	31	3/0/0	Bile duct bifurcation (II)	_	_	Yes
FR 1.9	23	79	3/1/1	Bile duct bifurcation (II)	Curative, but recurrence	_	Yes
FR 2/3.1	0	40	4/0/0	Common hepatic duct (I)	_	_	_
FR 4.1	2	82	4/0/*NA*	Common hepatic duct (I)	_	Yes	Yes
FR 4.2	0	57	3/0/*NA*	Bile duct bifurcation (II)	_	_	_
FR 5.1	0	72	2/1/0	Left hepatic duct convergence (III)	Exploratory	_	Yes
FR 6.1	0	68	2/0/0	Right hepatic duct convergence (III)	_	_	Yes
FR 7.1	0	43	3/0/0	Bile duct bifurcation (II)	_	_	_
FR 8.1	0	50	4/0/1	Common hepatic duct (I)	_	_	_
FR 8.2	1	38	4/1/*NA*	Common hepatic duct (I)	*NA*	Yes	Yes
FR 9/10.1	0	38	4/1/0	Common hepatic duct (I)	_	_	_
DE 1.1	0	41	4/0/0	Common hepatic duct (I)	_	_	_
DE 1.2	0	52	*NA*	*NA*	_	_	*NA*
DE 1.3	13	42	4/0/0	Common hepatic duct (I)	_	*NA*	Yes
DE 1.4	0	78	4/1/0	Common hepatic duct (I)	_	*NA*	*NA*
DE 1.5	11	18	*NA*	*NA*	Curative, but recurrence	Yes	Yes
PL 1.1	0	32	4/0/0	Common hepatic duct (I)	_	_	_
PL 1.2	1	57	4/0/0	Common hepatic duct (I)	Curative	_	Yes
PL 1.3	0	41	4/0/0	Common hepatic duct (I)	_	_	_
CH 1.1	0	58	3/0/0	Bile duct bifurcation (II)	_	Yes	_
CH 2.1	0	40	3/0/0	Bile duct bifurcation (II)	_	_	_
CH 2.2	0	48	2/0/0	Left hepatic duct convergence (III)	Curative	_	_
CH 2.3	0	55	4/1/0	Common hepatic duct (I)	_	_	_
CH 2.4	1	68	2/1/0	Left hepatic duct convergence (III)	Curative	_	Yes
CH 2.5	0	69	2/*NA*/0	Right hepatic duct convergence (III)	_	_	_
CH 2.6	0	52	2/0/1	Left hepatic duct convergence (III)	Curative	_	Yes
CH 2.7	8	37	2/0/0	Right hepatic duct convergence (III)	Curative	_	Yes
CH 3.1	0	74	3/0/0	Bile duct bifurcation (II)	Curative	_	Yes
CH 3.2	0	58	4/1/0	Common hepatic duct (I)	_	Yes	_
CH 4.1	0	62	4/1/1	Common hepatic duct (I)	_	_	Yes
Total (*n* = 38)	Median 0	Median 56			15	8	22

PEI: perendoscopic intervention; AE: alveolar echinococcosis; P/N/M: 'parasite, neighbor organs, metastasis' classification of AE lesions according to the Informal Working Group on Echinococcosis; PTBD: percutaneous transhepatic biliary drainage; NA: not available; BMZ: benzimidazole drugs

complications (7%); 26/38 patients had parasite-related bile duct stricture (Table 2). The procedure was successful, as defined by the resolution of the initial symptom within 7 days, in 95% of cases (122 PEIs). One patient had surgery and another one had PTBD after PEI failure. Other failures of PEI were successfully treated by a second PEI. Cholestasis and bilirubin levels dramatically decreased subsequently to PEIs (Figure 2).

During ERCP, biliary stones were reported in 29% (37/129) of procedures. Purulent bile was found in 18% (23/129) and antibiotics were used in 49% (63/129) of cases on the following days. Nine bacterial species were identified in the blood cultures and/or in the stent cultures. These bacteria were gram-positive cocci: *Enterococcus* sp. (*n* = 2) and *Enterococcus faecium* (*n* = 2); and gram-negative bacilli: *Escherichia coli* (*n* = 5), *Klebsiella oxytoca* (*n* = 1), *Klebsiella pneumonia* (*n* = 2), and *Proteus mirabilis* (*n* = 2). The median duration of antibiotics administration was 1 day before and 6 days after the PEI. An intensive lavage of the bile ducts was performed in 48% (62/129) of PEIs.

3.4. Biliary stenting

Stenting was performed in 30/38 patients (79%), a combination of stricture dilations and stent placements in 6 patients (16%), and sphincterotomy alone in 2 patients (5%) (Table 2); 139 plastic stents were inserted in 85 PEIs (median of 2 stents per PEI [1–4]). Median time between two PEIs according to the number of stents placed in the stricture section was 13, 16, 22, and 56 weeks for 1, 2, 3, and 4 stents, respectively. The median

Table 2. Indication, description, and outcome of the perendoscopic interventions (PEIs) performed in the 38 patients with alveolar echinococcosis (AE) included in the EndoscEchino European survey.

			Indications				*Endoscopic intervention*				*Resolution of stricture*	
Patient Number	*Year of first PEI*	*Number of PEIs*	*Jaundice without infection*	*Cholangitis*	*Pain*	*Surgery or PTBD failure*	*Only sphincterotomy*	*Sphincterotomy & stent placement (number of stents at first PEI)*	*Balloon dilation & stent placement (number of stents at first PEI)*	*Treatment of parasitic stricture*	*Duration of the PEI-based treatment (weeks)*	*Follow-up after the last PEI (weeks)*
BE 1.1	2012	3	Yes	–	–	–	–	Yes (2)	–	Success	31	62
FR 1.1	2005	9	–	Yes	–	–	–	Yes (2)	–	Success	477	25
FR 1.2	2000	3	–	Yes	–	Surgery	–	–	Yes (1)	No stricture	–	–
FR 1.3	2009	7	–	Yes	–	PTBD	–	Yes (2)	–	Failure	119	No follow-up
FR 1.4	2006	3	–	Yes	–	–	–	Yes (2)	–	Success	55	293
FR 1.5	2012	6	–	Yes	–	–	–	Yes (2)	–	Success	100	1
FR 1.6	2011	5	Yes	–	–	–	–	–	Yes (3)	Success	149	1
FR 1.7	2001	2	–	Yes	–	Surgery	–	Yes (1)	–	Still under endoscopic procedures	130	No follow-up
FR 1.8	2007	1	Yes	–	Yes	–	–	Yes (2)	–	No stricture	–	–
FR 1.9	2007	3	Yes	–	–	–	–	Yes (2)	–	Success	58	293
FR 2/3.1	2013	5	–	Yes	–	–	–	–	Yes (1)	Still under endoscopic procedures	69	No follow-up
FR 4.1	2004	3	–	Yes	–	PTBD	–	Yes (1)	–	Success	60	452
FR 4.2	2007	2	Yes	–	–	–	–	–	Yes (1)	Success	1	364
FR 5.1	2010	5	–	–	–	Surgery	–	Yes (1)	–	Success	103	50
FR 6.1	2013	4	–	Yes	–	–	–	–	Yes (1)	Still under endoscopic procedures	60	No follow-up
FR 7.1	2013	2	Yes	–	–	–	–	Yes (2)	–	Success	28	1
FR 8.1	2008	4	Yes	–	–	–	–	–	Yes (2)	Success	40	231
FR 8.2	2007	11	Yes	–	–	PTBD	–	Yes (1)	–	Success	154	102
FR 9/10.1	2014	4	–	Yes	–	–	–	Yes (1)	–	Success	35	1
DE 1.1	2010	5	–	–	Yes	–	–	Yes (1)	–	Success	240	1
DE 1.2	2009	4	–	–	Yes	–	–	Yes (1)	–	Success	45	1
DE 1.3	2012	3	–	–	Yes	–	–	Yes (1)		Success	6	1
DE 1.4	2004	3	–	Yes	–	–	–	Yes (1)	–	No stricture	–	–
DE 1.5	2003	3	–		–	PTBD	–	Yes (1)	–	Success	59	0
PL 1.1	2014	4	–	Yes	–	–	–	Yes (2)	–	Still under endoscopic procedures	36	No follow-up
PL 1.2	2011	2	–	Yes	–	Surgery	–	Yes (2)	–	No stricture	–	–
PL 1.3	2011	2	–	Yes	–	–	–	Yes (1)	–	No stricture	–	–
CH 1.1	2006	2	–	–	–	PTBD	–	Yes (1)	–	Success	30	382
CH 2.1	2011	4	Yes	–	–	–	–	Yes (1)	–	Still under endoscopic procedures	122	No follow-up
CH 2.2	2009	1	–	–	–	Surgery	Yes	–	–	No stricture	–	–
CH 2.3	2009	1	Yes	–	Yes	–	–	Yes (1)	–	No stricture	–	–
CH 2.4	2011	2	–	–	–	Surgery	–	Yes (1)	–	No stricture	–	–
CH 2.5	2011	2	Yes	–	–	–	–	Yes (1)	–	Success	26	128
CH 2.6	2007	4	Yes	–	–	Surgery	–	Yes (1)	–	No stricture	–	–
CH 2.7	1997	1	–	Yes	–	–	Yes	–	–	No stricture	–	–
CH 3.1	2011	2	Yes	–	–	Surgery	–	Yes (1)	–	No stricture	–	–
CH 3.2	2014	1	–	Yes	–	PTBD	–	Yes (1)	–	No stricture	–	–
CH 4.1	2014	1	Yes	–	Yes	–	–	Yes (1)	–	Failure	5	No follow-up
Total		129 (median 3)	14	16	6	14	2	29	7	19	Median 58.5	Median 50

PEI: perendoscopic intervention; PTBD: percutaneous transhepatic biliary drainage.

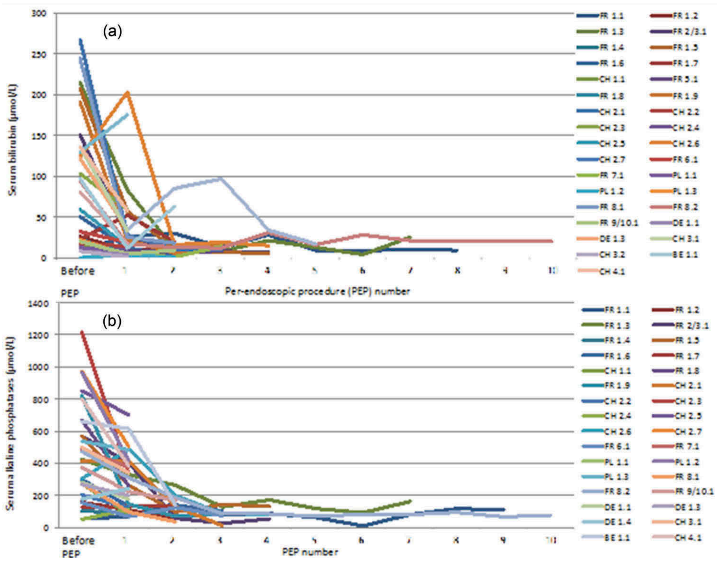

Figure 2. Course of serum bilirubin (µmol/L) (a) and serum alkaline phosphatases (µmol/L) (b), in those 33 and 31 patients respectively, treated by per-endoscopic intervention (PEI) who had a long-term biological follow-up. Serum bilirubin and alkaline phosphatases levels are given before first PEI; and after each PEI [1–10].

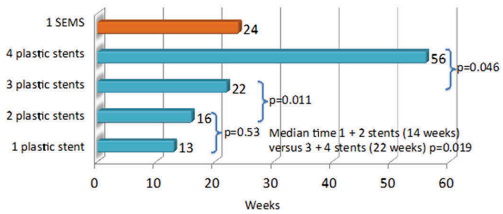

Figure 3. Median time (in weeks) between two per-endoscopic procedures according to the number of stents placed over the previous procedure. SEMS: self-expandable metallic stents. Statistical test: Mann-Whitney test.

time between 2 PEIs was significantly longer when 3 or 4 stents were put (22 weeks) than 1 or 2 stents (14 weeks) (p = 0.019) (Figure 3). Eleven self-expandable metallic stents (SEMS) (median: 1 stent per procedure [1–3]) were placed in 9 PEIs, fully covered stents in 9, and partially covered stents in 2. The median time between two SEMS placements was 24 weeks [0–130] (Figures 4 & 5).

3.5. Complications of the PEIs

Adverse events occurred in 22/129 PEIs (17%): 13 cholangitis (10 %), 7 acute pancreatitis (6 mild acute pancreatitis and one moderately severe acute pancreatitis) (5%), 1 duodenal perforation treated by surgery (0.8%), 1 retroperitoneal collection treated by radiological drainage (0.8%), and no death (Table 3). Thirty-nine PEIs were performed without intensive bile duct lavage during the procedure and/or without antibiotics; among them early cholangitis occurred in 9 (23%). Early cholangitis after PEI occurred 12 times; antibiotics had not been administered in 5, and intensive bile duct lavage had not been performed in 9 of these procedures.

Stent migration occurred in 11 procedures after a median time of 40 days [1–105] and stent obstruction in 5 procedures (in 4 cases there was only 1 plastic stent, obstruction occurred after a median time of 54 days [3–210]; in 1 case a single SEMS was plugged after 912 days) (Table 3). There were 2 early stent obstructions, both associated with the absence of intensive biliary lavage during the procedure. The occurrence of complications was not different according to the distribution by tertile of the PEI performance in the following time periods: before 2009, between 2009 and 2012, and after 2012. There was no difference either according to patient's age distribution by tertile at the time of PEI: 18–41 years, 42–67 years, and 68–86 years. The complication rate and the success rate of the PEIs were similar when PEIs were performed less (13% and 93%, respectively) or more (23% and 96%, respectively) than 3 years after AE diagnosis.

3.6. Long-term follow-up of AE patients and course of the disease after PEI

Two patients deceased within the follow-up period of time; cause of death was liver failure, not directly related to the biliary complications and/or the PEIs. Five patients were successfully treated by PEIs for biliary complications of radical/curative surgery (bile leakage, biliary fistula, stricture of biliary anastomosis) after a median of 2 PEIs and 45 weeks of treatment. Conversely, radical/curative

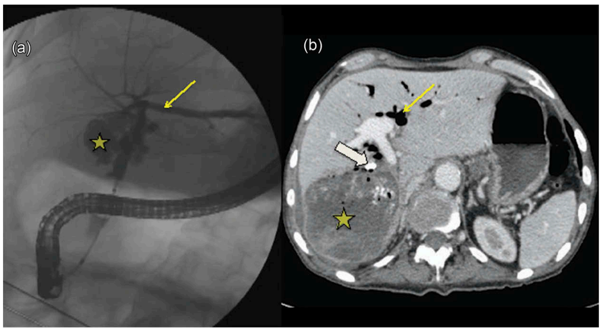

Figure 4. Imaging features of alveolar echinococcosis (AE) per endoscopic management. Cholangiography: communication between the parasitic necrosis (star) and bile ducts (small arrow) (a). Computed tomography: control of the drainage of the bile duct (small arrow: pneumobilia) with 2 plastic stents (large arrow) in a patient with AE and large parasitic necrosis (star) (b).

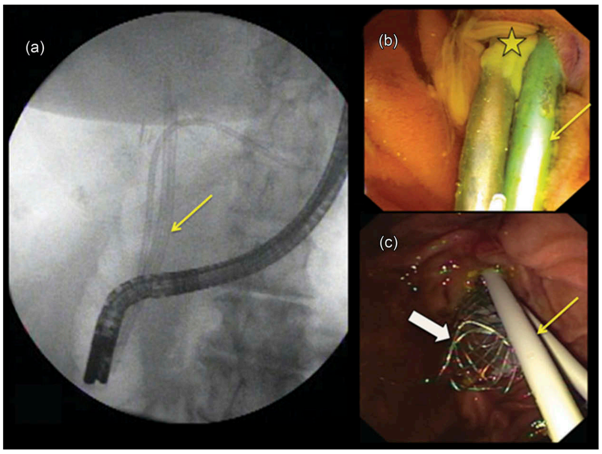

Figure 5. Endoscopic features of alveolar echinococcosic (AE) management with biliary stents. Fluoroscopy control during the per-endoscopic procedure: placement of 3 plastic stents (small arrow) in a patient with parasitic obstruction of the bile ducts (a). Endoscopic view: presence of purulent bile (star) before plastic stent (small arrow) exchange in a patient with AE complicated by cholangitis (b). Endoscopic view: placement of 2 plastic stents (small arrow) and 1 covered self expandable metallic stent (large arrow) in a patient with obstruction and stenosis of the common bile duct (c).

Table 3. Complications of the perendoscopic interventions (PEIs) performed in the 38 patients with alveolar echinococcosis (AE) included in the EndoscEchino European survey.

PEI complications (/129 PEIs)	Early complications (before day 30)	Late complications (after day 30)	Total
Cholangitis	12	1	13 (10%)
Acute pancreatitis	7	0	7 (5%)
Retroperitoneal collection	1	0	1 (0.8%)
Perforation	1	0	1 (0.8%)
Bleeding	0	0	0
Total	21 (16.2%)	1 (0.8%)	22 (17%)
Stent dysfunction (/94 stenting procedures)	Early dysfunction (before day 30)	Late dysfunction (after day 30)	Total
Stent migration	6	5	11
Stent obstruction	2	3	5
Total	8	8	16

surgery was made possible after 1 PEI in 3/38 included patients (1 duodenopancreatectomy, 1 hepatectomy with hepaticojejunostomy, and 1 liver transplantation, 60, 47, and 238 days, respectively, after the PEI); one of these patients, however, deceased within 7 days after surgery because of multiorgan failure. In a 4th patient, the primary aim of surgery subsequent to the PEI was curative but arterial involvement made radical surgery impossible and biliary diversion with hepaticojejunum anastomosis was instead performed 40 days after the first PEI.

In 26 patients with parasite-related bile duct stricture, the extent and location of the strictures were appropriately assessed and were treated by repeated PEIs. Bile duct patency was obtained in 19/26 patients (73%) with a median follow-up of 50 weeks after the last PEI [1 day–452 weeks], and there were no additional biliary complications during this period of time (Table 2). Perendoscopic treatment (all endoscopic techniques including repeated stenting, balloon dilation or both) was efficient after a median of 4 procedures [2–13] and a median time of treatment of 58.5 weeks [1–477]. Bile duct patency was not fully obtained at the end of the follow-up for 5/26 patients who were still treated by PEIs after 31 January 2015. Finally, endoscopic treatment for stricture failed in 2/26 patients: one needed surgical treatment because of recurrent biliary complications; the other deceased 5 weeks after the PEI because of liver dysfunction due to major parasitic involvement. Seven patients were treated with oral ursodeoxycholic acid after stent placement; none of them had a late stent obstruction at the end of follow-up.

4. Discussion

This retrospective survey of perendoscopic drainage in AE patients clearly shows that despite the low number of reported cases in the literature, most AE referral centers and several interventional endoscopy centers less involved in AE care management do use this technique routinely to treat biliary complications of AE. Our results show good efficacy and safety with a 95% overall success rate, resolution of bile duct stricture in 73% of cases, and 17% of nonmajor complications. Repeated biliary stenting associated with albendazole treatment may currently represent the best approach to prevent the mortality by AE which is frequently due to biliary/infectious complications of the disease, either early [7] or late [11] in the course of the disease. This survey also brings some light onto specific endoscopic technical aspects to efficiently prevent cholangitis and to achieve long-term dilation of the obstructed bile ducts in AE while ensuring the best quality of life to patients.

4.1. Design and limitations of the study

Because of its retrospective nature, this survey suffers obvious limitations: even if all AE referral centers positively answered, more than half of the cases could not be analyzed, because of local regulation or because technical details were missing in clinical reports for our analysis. In addition, it is likely that several interventional endoscopists were not reached by the information conveyed on the survey by professional or scientific societies. The low number of cases treated in some of the centers is due to both the fact that AE is a very rare disease and that most of them are treated in 'AE referral centers.' To ensure the homogeneity of recruitment and procedures (according to the technical recommendations of the European Society of Gastrointestinal Endoscopy), AE endemic areas such as Turkey and China were not covered by the survey [17,24]. China is the first endemic country in the world for AE; most cases are diagnosed at a very advanced stage, and Chinese surgeons have a huge experience of AE surgery, including major hepatectomy and allo- as well as auto-transplantation [25,26]. However, from the literature and our contacts with Chinese AE referral centers, we knew that nonsurgical interventions, whatever the type, were quite uncommon to treat AE biliary complications in China when our study was initiated; we thus decided to restrict the study to continental Europe.

4.2. Clinical outcome

Biliary complications of AE are frequent. Although expert opinions have repeatedly stressed that specific studies should be performed to support the use of alternative techniques to surgery to treat these biliary complications [11], data on PTBD are scarce and scattered [14], and until 2014, perendoscopic management of bile duct obstruction in AE had only been reported in a few case reports from Europe and small series of cases in Turkey [19–21,27]. In 2009, Ozturk et al. reported the follow-up of 13 patients treated with ERCP [27] with an overall disappointing efficacy, but only one patient was treated by biliary stenting at that time [27]. Our survey showed that 20 centers in Europe, including AE referral centers as well as digestive endoscopy centers dispersed over the European endemic area for AE, actually used PEI to treat AE biliary complications since 1997. Most of patients in our survey had an advanced stage of parasitic involvement of the liver, non-accessible to surgical resection. ERCP was performed within the first year after diagnosis in two-thirds of patients, and cholangitis, jaundice, and biliary pain were the presenting symptoms that required PEI, sometimes in emergency situations. This confirms that biliary complications are frequently observed in the immediate post-diagnosis period and, as the efficacy and safety of the procedure were excellent, proper use of PEI might reduce the excess of mortality observed in that period [7]. Our survey also showed that PEIs could alleviate the 'late' biliary complications in 96% of the cases, as successfully as the 'early' ones, and thus greatly improve the prognosis of such cases [11] and quality of life since it avoided the discomfort of the external drain often necessary after PTBD. Prevention of secondary biliary cirrhosis which is the direct consequence of chronic bile duct obstruction/infection and may lead to liver failure and to hepatocarcinoma is also an important issue and should be among the main benefits of the PEIs on the long term. Finally, because PEIs were efficient to treat the biliary complication as a 'neoadjuvant' therapy, radical resection of the lesion could even be reconsidered in a few cases.

4.3. Complications and morbidity

Our survey showed 17% of complications; which may appear rather high compared to PEIs performed for other indications

(7%) [28,29]. The difference is clearly related to the frequent occurrence of post-interventional cholangitis, far more frequent than in other indications (10% versus 1.44% in a recent meta-analysis, respectively) [28]. Other early complications of PEIs in AE patients were not different from those observed in other indications [17,28]. In symptomatic cases, AE is diagnosed after years of slow progression of the parasitic disease, associated with progressive invasion and obstruction of the bile ducts and in some cases intrahepatic stones. Spontaneous degeneration of the parasite due to a partially effective immune response often results in a central cavity, filled with necrotic debris, which eventually communicate with the obstructed/dilated biliary tree, and bacterial superinfection is nearly always present in these advanced cases [5]. This is a rather unique condition, which explains the frequency of cholangitis after the PEIs, as was also observed after AE surgery [5,11,13]. The recurrence of cholangitis often associated with bacteremia and septic shock, leading to repeated antibiotic treatments and operations, was the main reason for recommending to avoid palliative operations in AE patients [5,8]. From the data obtained in this survey, despite the limited number of patients which prevents a proper statistical analysis, the main preventive measure to reduce the risk of cholangitis, which should be recommended to interventional endoscopists, is an intensive biliary duct lavage during the procedure with full removal of stones and parasitic debris. Even though concomitant use of antibiotics seemed to be less crucial for the outcome, systematic use of systemic antibiotics, if possible before and at least one week after the PEI, secondarily adapted to the infectious organisms found in bile and/or blood culture, could help for a better prevention of post-PEI cholangitis. In the survey, there were no more complications in older patients, who can thus be treated safely by PEIs.

4.4. Technical aspects

To endoscopists, AE associates a benign condition with long survival time and the involvement of both hilum and proximal bile ducts. SEMS were used in only a few cases in our survey; the use of SEMS, however, seemed efficient to obtain bile duct patency, with a longer time interval between two PEIs than with plastic stents (Figure 3). Removable covered SEMS are used to treat biliary stricture or biliary leaks of the distal common bile duct after surgical injury or in chronic pancreatitis [17]. Such covered stents cannot be used in the upper biliary tree, because they may cause occlusion of side branches, which explains the relatively infrequent use of these stents in AE. Insertion of multiple plastic stents side by side may resolve this technical issue, as they are completely removable and do not obstruct the bile duct bifurcation; however, stent dysfunction is more frequent with plastic stents and needs several repeated procedures [14,17]. In our survey, plastic stent placement was indeed the most common procedure. Interestingly, time interval between two PEIs positively depended on the number of stents placed in the stricture section at previous procedure; enlargement of the stricture using hydrostatic balloon dilations allowed several stent placement and thus enhanced the procedure. We may note that most endoscopists inserted 1 or 2 stents at initial procedures (Table 2) then tended to increase the number of stents when they repeated the procedure, and this resulted in better efficacy. Perendoscopic treatment of 7 patients including 6 patients with bile duct strictures treated by stent placement followed by several balloon dilations without new stent placement were recently reported [30]; comparison of the 4 patients with more than 3 months-follow-up after last dilation to those with repeated stenting in our survey shows that repeated dilations are associated with an increased number of procedures (9 on an average) and duration of treatment (more than 150 weeks). The nature of bile duct stenosis in AE, characterized by dense, acellular, irreversible fibrosis [31] may explain the difficulty of balloon dilations alone in obtaining sustained bile duct patency. In AE, because of the chronic infection of the biliary tract which produces sludge and parasitic/necrotic plugs, systematic numerous stent placement and early stent exchange should be proposed to prevent plastic stent dysfunction. To be widely applicable, such attitude should however be supported by a prospective study. Although treatment with oral ursodeoxycholic acid or intermittent antibiotic treatments were inefficient to avoid stent obstruction in the literature in case of pancreatic cancer [32], the additional advantage of ursodeoxycholic acid treatment, which was used in 6 centers, should be further studied by controlled trials in AE, since the two conditions are markedly different.

5. Conclusion

This European multicenter survey is the biggest series so far on perendoscopic biliary drainage in patients with AE and shows that this technique is used routinely to treat biliary complications with good efficacy and safety. Cholangitis is most frequently seen after PEI for AE than for other indications. Intensive lavage of the bile ducts during the procedure in combination with antibiotics should prevent cholangitis. Insertion of multiple plastic stents (at least three) prolongs the time intervals before stent occlusion and leads to effective and prolonged patency of obstructed bile ducts.

Key issues

- Perendoscopic interventions (PEIs) with bile duct stenting are routinely used in Europe for the treatment of AE biliary complications.
- Resolution of the initial symptom within 7 days was obtained in 95% of cases.
- Stenting was performed in 30/38 patients (79%).
- The median time between stent placements was significantly longer when 3 plastic stents or more were placed.
- In patients with parasite-related bile duct stricture, repeated perendoscopic interventions over an average of 14 months of treatment was efficient to obtain long-term bile duct patency.
- Intensive biliary duct lavage during the procedure and full removal of stones and parasitic debris may reduce the risk of cholangitis which represents a frequent complication of the perendoscopic interventions in this indication.

Acknowledgments

The authors acknowledge the physicians, endoscopy and clinical research nurses who have participated in the care management of the AE patients and/or in the recruitment of cases: Beat Müllhaupt, and Christoph Gubler

(Zurich, Switzerland) who unfortunately could not include their cases in this survey; Eric Delabrousse, Olivier Sylvant, Marie-Elizabeth Deroze, Coralie Cuenin, Bérénice Vernet, Nathalie Lantoine, & Maryse Courbet (Besançon, France), Patrizia Künzler (St Gall, Switzerland), Wojciech Wołyniec and Wacław Nahorski (Gdynia, Poland) and Florent Coulon (Besançon, France).

Funding

This paper was not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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8 Conclusion

8.1 Discussion

8.1.1 Diagnostic ERCP for characterization of indeterminate biliary stenosis

Despite the development of less invasive diagnostic techniques, in 2021 ERCP remains essential for the diagnosis of biliary stenosis after a work-up including laboratory blood tests and cross-sectional imaging examinations: CT scan and MRCP. The continuation of the diagnostic workup by an EUS with FNA for cyto and histopathological diagnosis, of which the risk of complication is much lower than that of the ERCP will be considered in the presence of a tumor nodule identified beforehand on the examinations of cross-sectional imaging. EUS requires deep sedation with Propofol and often the presence of an anesthesia team for general anesthesia with tracheal intubation. The voluntary non-performance of an ERCP at the same time of EUS is only justified in the presence of a clearly identified tumor nodule and in the absence of the need for a therapeutic ERCP for biliary drainage in the cases where resection surgery (pancreatoduodenectomy) is planned with a short delay.

Because of the risks of the ERCP, except in an emergency such as a severe sepsis due to a cholangitis, the indication for a first diagnostic ERCP in the context of the biliary stenosis assessment of undetermined origin must be confirmed by multidisciplinary advice: hepato-gastroenterologists, surgeons, oncologists, radiologists and pathologists specializing in hepatobiliary and pancreatic pathologies. In our facility; University Hospital of Geneva, the files of these patients are formally discussed during a specialized forum (CHBP = hepato-biliary and pancreatic diseases center) before carrying out the diagnostic ERCP then reviewed with the results of the endoscopic assessment and the results of the analysis of the samples.

The simplest techniques to optimize the diagnostic yield of the first ERCP during the assessment of a biliary stenosis should be implemented: during cytological brushing, sending bile collected in the biliary duct after brushing and using the same device significantly increases diagnostic sensitivity [31]. The use during the ERCP of a guided-wire biopsy forceps is a safe procedure to obtain histological samples.

The processing of samples taken during the ERCP and their transport to the pathology laboratory requires rigorous logistics, in particular for cytopathological diagnosis. In particular, fresh bile samples taken after brushing must be sent immediately to the cytopathology laboratory [30].

In case of indeterminate biliary stenosis at the end of the assessment, performing a cholangioscopy during the second ERCP with the use of a single-use cholangioscope handled by a single operator (SOC) with the practice of directed biopsies makes it possible to increase diagnostic accuracy and modify patient management [57]. A recent prospective, randomized multicentric study [78] evaluated the results of SOC compared with standard ERCP with endobiliary brushing as a first-line diagnostic tool for biliary stenosis detected with MRI cholangio. The sensitivity of SOC-guided biopsy was significantly higher than that of ERCP-guided brushing, respectively (68.2% vs. 21.4%, $p < 0.01$). With the SOC, the sensitivity of visualization was 87.1% vs 65.5% (p 0 0.05) and the overall accuracy were significantly higher: 95.5% vs 66.7%, ($p = 0.02$) with no significant difference in specificity, positive predictive value and negative predictive value. The use of SOC as a first-line treatment for the characterization of biliary stenosis does not appear to be relevant at this time in the absence of studies evaluating the cost-effectiveness ratio of this attitude.

However, up to now, our arsenal still lacks an ideal diagnostic technique that will allow very high diagnostic sensitivity to be obtained while maintaining the excellent specificity of cytology and histopathology to avoid surgical management of indeterminate biliary stenosis. The combination of SOC and pCLE has recently been proposed to improve targeting of virtual biopsies [79]. A cost-effectiveness evaluation of this technique would be necessary before using it routinely given the very high cost of single-use devices to be used. The increase in diagnostic performance (accuracy) could be obtained with techniques that are easier to perform without a sophisticated endobiliary device and from a bile sample taken during the first ERCP. The high concentration of extracellular vesicles in bile samples from 30 patients: 15 malignant stenosis and 15 chronic pancreatitis appeared (significantly) higher in cases of malignant stenosis [80]. The implementation of a proteomic technique from a bile sample (liquid biopsy) by the same teams appears to be a promising avenue. In fact, the identification in the bile of proteins identified as biomarkers of malignancy combined with the assay of serum Ca 19.9 made it possible to obtain a diagnostic accuracy of 90%.[81]. The use in daily clinical practice of this liquid biopsy technique remains to be implemented.

8.1.2Therapeutic ERCP for benign biliary stenosis

8.1.2.1 cSEMS

For the treatment of benign biliary stenosis, the use of cSEMS appears to be an advance by making it possible to obtain from the outset an optimal calibration of the stenosis and to limit the number of ERCP. In the study presented as publication 4 [77], cSEMS could be left in place 6 months against 3 at 4 months for plastic stents, in particular for distal biliary stenosis of chronic pancreatitis and post-cholecystectomy.

The use of cSEMS for the treatment of benign stenosis has been the subject of an international multicenter prospective trial with large numbers of patients [82]. The cSEMS change was recommended every 4 to 6 months for 6 months for patients with orthotopic liver transplantation (n = 42) and 1 year for patients with chronic pancreatitis (n = 127) or a complication of a laparoscopic cholecystectomy (n = 18). Clinical success: resolution of the stenosis, was obtained in 76.3% of patients with a mean follow-up of 20.3 months. In the absence of early migration, resolution of the stenosis was achieved in 90.5% of patients with chronic pancreatitis, 88% of patients with orthotopic liver transplantation, and 90.9% of patients with a complication of laparoscopic cholecystectomy.

In order to facilitate endoscopic extraction, the use of cSEMS equipped with a lasso located at the proximal end of the device was proposed in a study involving 23 patients [83]. Thirty-nine cSEMS were placed and removed easily and without complications. To our knowledge, no prospective study about this topic is available. Moreover, in common practice, removal of a cSEMS with a snare (type polypectomy snare) or a foreign body forceps is rarely difficult.

In addition, one of the main complications secondary to the use of cSEMS is the risk of early duodenal migration of the device. Several teams have reported the use of special design cSEMS or dedicated devices to limit the risk of complications. The use of a cSEMS with an anti-migration design studied in a cohort study did not show efficacy with a migration rate of 31% [84]. On the other hand, it was suggested that the use of an anchor of the cSEMS by a plastic stent could significantly reduce the migration rate: 6.3% vs 41.2% in a study of 32 patients [85]. Currently due to the low level of evidence, these devices or stent with a particular design are not used in practice.

8.1.2.2 Use of plastic stent or SEMS?

This comparison between the two types of stents including non-neoplastic stenosis without distinction of their etiology was the subject of a systematic review and meta-analysis published in 2017 [86]. Twenty-two studies with a total of 1298 patients were considered. There was no difference between the two types of stents in terms of resolution of the stenosis, recurrence of the stenosis and the occurrence of side effects. The number of ERCPs was significantly lower (- 1.71 (2.33 - 1.09) with the use of a cSEMS.

Three randomized controlled trials compared the efficacy and safety of treatment with cSEMS vs. multiple plastic prostheses fort the treatment of biliary stenosis after liver transplantation [87 65 88] and were taken into account in a meta-analysis [89]. There was no difference noted between the two procedures in terms of clinical success in stenosis resolution and recurrence. On the other hand an advantage was shown for the number of procedures and the duration of the treatment with on average a difference of 1.69 ERCP 95% CI, 1–2.39; p <0.00001) and a mean difference of 40.2 days 95% CI, 3.9–76.4; p = 0.03) in favor of treatment with cSEMS [89].

In the indication biliary stenosis following chronic pancreatitis, a randomized study showed no difference between the two types of stents during a treatment lasting 6 months with a success rate at 2 years of resolution of the stenosis of 90% (cSEMS) and 92% (plastic prostheses), the migration rate was similar: 10 and 7%.[90]

The ESGE guidelines recommend the placement of a stent as a first-line treatment in the event of fibrous stenosis of the main bile duct in chronic pancreatitis without indicating a preference for a type of stent: SEMS vs multiple plastic stents [91]

An important point is the contraindication in relation with the site of the benign stenosis for using cSEMS. Indeed, except in particular cases, a cSEMS is not to be use for a stenosis located above the common bile duct to prevent obstruction of a part of the biliary tree. Moreover, it is preferable to respect a 2 cm interval between the proximal edge of the cSEMS and the hepatic bifurcation to prevent an obstruction of the hepatic ducts with a hyperplastic granulomatous inflammatory reaction above the cSEMS. In addition, if no cholecystectomy was previously performed, the proximal edge of the cSEMS is to be located under the cystic biliary bifurcation to prevent a cholecystitis.

Concerning biliary stenosis after hepatic transplantation, in our experience, treatment remains largely based on stenting by the placement of plastic prostheses. In particular biliary stenosis occurring after liver transplantation with a living donor occurs most often in bile ducts too small for the placement of cSEMS even 6 or 8 mm in diameter. The continued preferential use of plastic prostheses and the rare and risky use of cSEMS in this indication was reported in a recent review [92]. For the treatment of biliary stenosis following liver transplantation from deceased donors, our initial experience with the use of cSEMS [93] was confirmed and a recent meta-analysis confirmed the similar efficiency of the two types of stenting with an advantage for cSEMS by performing fewer ERCPs [94]. However, the use of cSEMS in this indication is conditioned by the anatomy of the surgical biliary anastomosis. To place safely a cSEMS a distance of at least 20 mm should be maintained between the upper edge of the stenosis and the upper biliary convergence to avoid the occurrence of inflammatory tissue proliferation in the right and left bile ducts. In addition, the occurrence of biliary stenosis above the superior biliary convergence is frequent in biliary stenosis of ischemic origin due to hepatic artery stenosis and constitutes a contraindication to the use of cSEMS. The use of resorbable stent put in place during surgery has been reported in a pilot study in liver transplant patients with living donors [95]. New biodegradable stents are under development [96].

8.2 Challenges on ERCP practice in 2021

8.2.1 Therapeutic EUS

In 2021, advances in therapeutic EUS allow the use of this technique in the event of failure of ERCP for drainage of malignant biliary stenosis due to digestive stenosis or tumor invasion of the major duodenal papillae by performing hepatico-gastrostomy or choledoco-duodenal drainage using a lumen-apposing metal stent [97]. However, this technique cannot, in the current state of knowledge, replace ERCP, either for the diagnosis of indeterminate biliary stenosis or for the treatment of benign biliary stenosis.

8.2.2 ERCP learning, teaching and skill maintenance

The biggest challenges today for interventional endoscopy is the resolution of difficulties in the ERCP training of junior physicians and for physicians qualified in interventional endoscopy to perform a sufficient number of ERCPs to maintain their skills.

Before starting an ERCP training, the prior competence in standard endoscopy: gastroscopy, colonoscopy, standard endoscopic mucosal resection and polypectomy, endoscopic hemostasis, foreign body extraction is required. Due to the risks of ERCP, the initial learning of ERCP on a simulator should be considered before beginning to experience coaching in patients. Three randomized studies were published, two Asian studies and one US study concluded to the statistically significant benefit of the initial training on a mechanical simulator in particular for the retrograde cannulation time of the duodenal papilla. [98 99 100]. In particular in the study by Liao et al [99], the result of training days on a mechanical simulator was evaluated on the papilla cannulation time of the students during the first three months of their practice in patients. This time was significantly shorter aOR =2.89, (CI 95% = 2.21 - 3.80) (P <0.001) among the students (n = 16) who had benefited from the training on the simulator. There was no statistical difference for the group that received only one day of training versus the group that continued with mechanical simulator training every two weeks for 3 months. We have at the University Hospital of Geneva (HUG) thanks to the Swiss Foundation for Innovation and Training in Surgery (SFITS) a computerized simulator (Simbionix ®) available for learning digestive endoscopy and particularly ERCP. However, data from the literature [101] and our experience are not in favor of providing this type of learning for ERCP unlike the initial learning of colonoscopy [102].

A sufficient duration of training under the responsibility of a mentor in a center with a sufficient volume of ERCP is required. For ESGE, a training of one to two years is required. The number of diagnostic and therapeutic ERCPs carried out by the physician in training independently but under supervision during this training period must be 100 including 75 sphincterotomies (biliary or pancreatic), 30 stent placements (plastic / metal), 40 stones extractions (balloon, Dormia basket, and lithotripsy) [106] For the Swiss Society of Gastroenterology (SSG-SGG), the requirements of the postgraduate program are similar: 100 ERCP, including 50 with sphincterotomy, 25 stone extractions, 25 drainages (stents, pig-Tail catheter, nasobiliary probes) [107] . There is currently no theoretical and practical postgraduate training organized in Switzerland and it would require a very heavy investment to train 1 or 2 specialists in interventional endoscopy per year. However, this type of French language training does exist: Interventional endoscopy Inter-University Diploma (DIU) from the University of Paris [108]. This 2-year training course includes theoretical training by international experts in interventional endoscopy, practical teaching modules on animal models allow practical knowledge to be checked, and the practical training record attests to the training acquired in companionship. with a training supervisor recognized by the profession. The animal model is closed to the Erlangen easy trainer [103],

upper visceral porcine organ including esophagus, stomach, duodenum, liver and biliary tract are prepared in a dedicated box. With a mobile X-ray unit and a usual duodenoscope, ERCP can be performed using all the devices with a realistic way. This training is open to European gastroenterologists. During the last 5 years, 3 gastroenterologists working in French-speaking Switzerland have benefited from it. About fifteen gastroenterologists obtain the French diploma each year.

In addition, an essential point is to ensure that doctors qualified for ERCP can work in a center with sufficient volume of ERCP to maintain their skill. For more than two decades, considering the risk of complications of diagnostic ERCP which was around 5% even without an associated therapeutic procedure, ERCP has been advantageously replaced by non-invasive MRCP and by the much less invasive EUS. Thereby the volume of ERCP practiced in each center has sharply decreased (450 / year at HUG), limiting training possibilities. The practice of less than 200 ERCP per year is associated with a higher complication rate. Particularly in a multicenter observational study, in multivariate analysis, the rate of post ERCP pancreatitis and post sphincterotomy hemorrhage was significantly higher in endoscopists who performed less than 200 ERCP per year [104].

These considerations regarding initial training in ERCP and maintaining a sufficient volume of activity should be considered in order to retain specialists with a high level of competence in diagnostic and therapeutic ERCP.

8.3 Research perspectives

The venue from March 2022 of a third colleague practicing interventional endoscopy should save us time to develop several lines of research in addition to clinical activity and teaching.

- **The creation of an ERCP database** performed at the HUG is a priority. This database should be focused on the one hand on current and future diagnostic procedures and on the other hand on therapeutic ERCP in particular stent implantation.
- **The implementation of liquid biopsies** (in bile obtained during ERCP) to improve the diagnostic performance in indeterminate biliary stenosis appears as a major advance. Accordingly, several bile biomarkers including proteins [81], metabolites, microRNAs and DNA methylation [105] have recently emerged as a promising option for the molecular diagnosis of malignant biliary stenosis.
- The ongoing active participation in multicenter studies focusing on hepatobiliary situations that require specific and challenging endoscopic interventions, is a priority for our community and the institution in terms of international visibility. For example, the place of diagnostic cholangioscopy by SOC is strongly accepted [57], but whether it should performed at first or

second intention in the diagnostic process has not been determined yet. Our **active participation in the international multicenter study SPYGLASS** under the aegis of SFED (French Society of Digestive Endoscopy) is scheduled for 2022.

Multicenter prospective study.

Comparison, in terms of diagnostic performance, of two strategies for exploring indeterminate biliary stenosis, retrograde cholangioscopy by Spyglass DS as first-line (new strategy studied: "study" group) versus second-line after failure of conventional techniques ("strategy") usual / current ":" control group ").

- The endoscopic management of anastomotic biliary strictures following living donor liver transplantation is currently a technical challenge due to complex biliary anastomoses and anatomical variations in biliary anatomy. The experience of transplant centers in western countries is limited due to the relatively scarce number of liver transplantation with living donors. Therefore, we plan to **elaborate a prospective study in order to standardize the endoscopic treatment of biliary stenosis following living donor liver transplantation**

9 Acknowledgments

First of all, I would like to express my gratitude to all colleagues who led and participated in the studies. In particular colleagues from the Grenoble University Hospital: Michèle Fior-Gozlan, Jean Auroux, Gaël S Roth, Vincent Leroy and members of the SFED: Ulriikka Chaput, Stéphane Koch including Jean Boyer, Frédéric Prat and Thierry Ponchon, heads of the DIU of interventional endoscopy for the trust they placed in me.

Finally, I really want to thank my colleagues and friends Jean Louis Frossard, Laurent Spahr, Nicolas Goossens and Emile Giostra for the welcome they gave me and the kindness towards me which allowed me to integrate myself into the team since my arrival at the University Hospital of Geneva. The advices they gave me were very helpful for this job. Without forgetting Caroline Bastid who, thanks to her kindness and her organizational skills, has been able to adjust my schedule in recent months.

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