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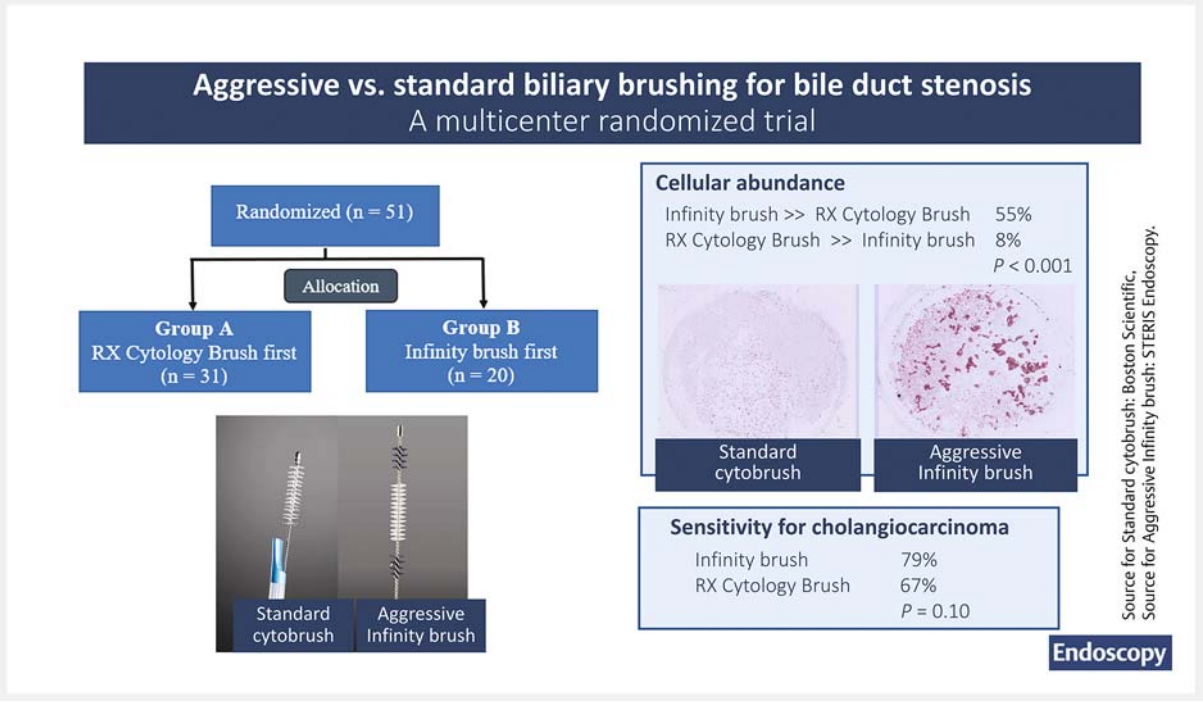
Multicenter randomized trial comparing diagnostic sensitivity and cellular abundance with aggressive versus standard biliary brushing for bile duct stenosis without mass syndrome

GRAPHICAL ABSTRACT



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ABSTRACT

Background The diagnosis of cholangiocarcinoma in patients with a biliary stricture without mass syndrome can be obtained by biliary brushing with a sensitivity of ~50%. We performed a multicenter randomized crossover trial comparing the aggressive Infinity brush with the standard RX Cytology Brush. The aims were to compare sensitivity for cholangiocarcinoma diagnosis and cellularity obtained.

Methods Biliary brushing was performed consecutively with each brush, in a randomized order. Cytological material was studied with blinding to the brush type used and order. The primary end point was sensitivity for cholangiocarcinoma diagnosis; the secondary end point was the abundance of cellularity obtained with each brush, with cellularity quantified in order to determine if one brush strongly outperformed the other.

Results 51 patients were included. Final diagnoses were cholangiocarcinoma (n = 43; 84%), benign (n = 7; 14%), and indeterminate (n = 1; 2%). Sensitivity for cholangiocarcinoma was 79% (34/43) for the Infinity brush versus 67% (29/43) for the RX Cytology Brush ($P = 0.10$). Cellularity was rich in 31/51 cases (61%) with the Infinity brush and in 10/51 cases (20%) with the RX Cytology Brush ($P < 0.001$). In terms of quantification of cellularity, the Infinity brush strongly outperformed the RX Cytology Brush in 28/51 cases (55%), while the RX Cytology Brush strongly outperformed the Infinity brush in 4/51 cases (8%; $P < 0.001$).

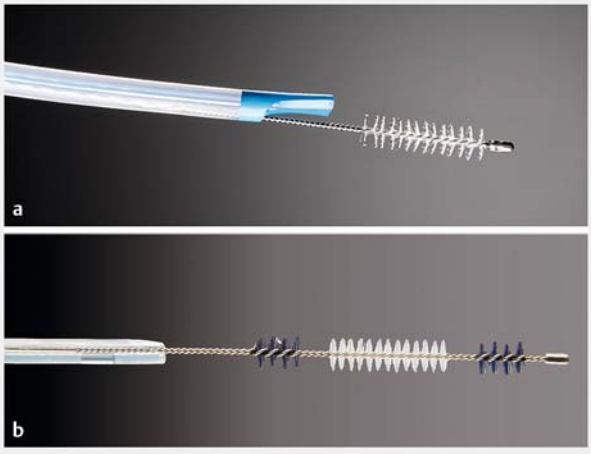
Conclusions This randomized crossover trial showed that the Infinity brush is not significantly more effective than the RX Cytology Brush for biliary stenosis without mass syndrome in terms of sensitivity for cholangiocarcinoma diagnosis, but does offer a significantly higher abundance of cellularity.

Introduction

Cholangiocarcinoma is the most frequent cause of jaundice in patients with intraductal bile duct stricture [1]. Bile duct drainage is often the first-line treatment in the management of such patients, but physicians should rapidly consider whether chemotherapy and/or biliopancreatic surgical resection are appropriate [2–5]. Prior to such treatment, cholangiocarcinoma cytopathological characterization must be determined, because 5%–25% of indeterminate bile duct stenoses are benign and 3%–7% of patients who undergo surgery for a suspected malignant bile duct stenosis have a benign disease [1, 6–8].

Endoscopy plays a paramount role in cytopathological diagnosis. If there is mass syndrome, cytopathological diagnosis is easy to obtain by endoscopic ultrasound-guided fine needle biopsy (EUS-FNB) [9, 10]. Unfortunately, where biliary stricture occurs without mass syndrome, the diagnosis of cholangiocarcinoma is difficult to obtain by biliary brushing during endoscopic retrograde cholangiopancreatography (ERCP), with approximately 30%–60% sensitivity [1, 11, 12]. Among the alternatives developed to overcome the low sensitivity of biliary brushing for cholangiocarcinoma, new generation intraductal cholangioscopy (SpyGlass DS Direct Visualization System; Boston Scientific Corporation, Marlboro, Massachusetts, USA) with biopsies seems to be the most effective technique, with a higher, but not perfect, 69%–74% diagnostic sensitivity [12–14]. However, organizing such a time-consuming procedure as the first-line treatment in routine practice is complex and expensive. Intraductal cholangioscopy is also not available in every endoscopy unit. Therefore, we wanted to explore ways of increasing the sensitivity of biliary brushing, with the aim of determining whether this sampling method could continue to be used as the first-line procedure.

A more aggressive brush with a larger diameter that comprises two rows of stiffer bristles surrounding standard flexible bristles has recently been developed (▶ **Fig. 1**). In the very few studies published on the Infinity brush (US Endoscopy, Steris Healthcare, Ohio, USA), sensitivity for cholangiocarcinoma of



▶ **Fig. 1** Photographs of the biliary brushes used in the study: **a** the standard RX Cytology Brush (Source: Boston Scientific); **b** the more aggressive brush (Source: STERIS Endoscopy).

approximately 85 % and accuracy of 87 % has been reported [15, 16]. In contrast, in an initial randomized controlled trial (RCT) on 60 patients with biliary stricture, in which malignant strictures were almost all related to pancreatic cancer and not to biliary duct cancer, the Infinity brush did not show any advantage in terms of sensitivity compared with the conventional cytology brush [17].

To determine which type of brush obtains the largest quantity of cytological material, we performed a multicenter randomized crossover trial comparing the aggressive Infinity brush with the standard RX Cytology Brush (Boston Scientific Corporation). The primary end point was the sensitivity for cholangiocarcinoma diagnosis, and the secondary end point was cellular abundance, as evaluated by: (i) a four-stage classification of the cellularity obtained, and (ii) blind determination of a strong outperformance of one brush compared with the other.

Methods

This multicenter randomized crossover trial was conducted in 12 expert tertiary endoscopy centers from 1 April 2020 to 1 October 2021. All investigators were members of a French taskforce of gastroenterologists working in digestive endoscopy (Groupe de Réflexion et d'Action des Praticiens Hépatogastroentérologues en Endoscopie Digestive [GRAPHE]). The work was sponsored by the French Society of Digestive Endoscopy. Written informed consent for the ERCP procedure was obtained from all patients. The study was carried out in accordance with the Helsinki Declaration and was approved by the Ouest I ethics committee of the University Hospital of Tours, France (number 2019T2–29 DM) and the French National Agency for the Safety of Medicines and Health Products (ANSM 2019-A02618–49). It followed the recommendations of the STARD and CONSORT statements. All authors had full access to the study data.

Patients

The study included all consecutive patients aged ≥ 18 years who were referred to one of the participating expert tertiary endoscopy centers to undergo an ERCP for a bile duct stenosis. Patients with a significant tissue mass (≥ 1-cm well-organized mass) that could easily be punctured by EUS-FNB were not included. Exclusion criteria were predefined as follows: nonaccessibility to the bile duct, coagulation disorders, treatment with clopidogrel, or pregnancy.

Groups and randomization

Computer-generated randomization assignments establishing the order of brush use were placed in sealed envelopes to be opened locally during the ERCP procedure when the patient matched the inclusion criteria. Both brushes were used in each patient, with the RX Cytology Brush used first in group A, and the Infinity brush used first in group B.

ERCP procedure

All of the ERCP procedures were performed by expert endoscopic physicians, who annually perform more than 200 ERCP procedures each. The following duodenoscopes were used: (i) the Olympus TJF-Q180 V and TJF-Q190 V (Olympus Europe Inc., Hamburg, Germany); (ii) the Fujinon ED-580XT (Fujifilm France [Medical Systems], Asnières, France); and (iii) the Pentax ED 34-i10 T2 (Pentax Europe, Hamburg, Germany). After deep bile duct catheterization with a guidewire and biliary sphincterotomy had been performed, biliary brushing was performed using the two brushes according to a randomized assignment. Antibiotic prophylaxis was almost always given for hilar or perihilar stenoses, according to recommendations [18]. The endoscopist involved in each procedure performed either 6– or 8-mm balloon dilation of the stenosis before brushing, based on his or her own routine.

After the envelope had been opened to reveal the patient's allocated group, the assigned first brush was moved in and out of the catheter about 10 times in back and forth movements through the stricture. Three smears were made on slides and the brush was cut and placed, along with the bile gently flushed from the catheter, into formalin or CytoLyt solution (depending on the center). The same procedure was systematically repeated with the second brush, with three more smear slides prepared and the brush and flushed bile placed in a second sampling pot.

Another sampling method was then performed (EUS-FNB, intra-choledochal biopsies taken using fluoroscopy or intraductal SpyGlass DS cholangioscopy guidance) to maximise the chances of obtaining cytopathological characterization of the stenosis.

Histological preparations and analysis

The cytopathological techniques used for each brush were conventional cytology (smear with Papanicolaou or May Grünwald–Giemsa staining), liquid-based cytology, or both, depending on the practice of the pathology department. In order to allow comparison, the same cytopathological techniques were used

for both brushes for each patient. The tissue fragments obtained by EUS-FNB and intracholedochal biopsies, which had been formalin fixed and delivered to the cytopathology unit within 2 days, were paraffin embedded and stained with hemateine-eosin-safran (HES) stain.

A first examination of the cytological samples obtained by biliary brushing was performed in each center, along with a centralized examination performed by one expert physician in pancreaticobiliary pathology (A.C.), in a blinded manner with regard to the type of brush and the order of use. Specimens were however evaluated with the knowledge that both brush samples had been obtained from the same patient.

The following pathological criteria were assessed:

- quantification of the cellularity of each brush into four grades: poor; moderate; rich; rich with plenty of cell clusters
- determination of a strong outperformance of one brush compared with the other, in terms of the cellularity obtained
- biliary stricture characterization following the standardized terminology for pancreaticobiliary cytology defined by the Papanicolaou Society of Cytopathology Guidelines [19].

Data collection and post-procedure management

We collected patient characteristics, biliary stricture location, and brushing characteristics (complete or incomplete owing to technical difficulties), and recorded any post-procedural biliary drainage failures and complications.

Complications and deaths were recorded for both groups. Morbidities due to ERCP were defined and graded according to the modified 1991 consensus guidelines [20]. Repeat and/or alternative procedures were performed in patients where false-negative diagnoses were suspected. Additional physical and computed tomography (CT) examinations were performed at least 18 months after the procedure on patients who were negative for malignancy.

End point definitions

The primary end point was sensitivity for cholangiocarcinoma diagnosis obtained by biliary cytopathological examination, corresponding to category V (suspicious for malignancy) and category VI (malignant) in the Papanicolaou Society of Cytopathology Guidelines [19], EUS-FNB, or surgical resection, and/or based on tumor evolution after more than 18 months of follow-up.

The secondary end points were to quantify the cellularity obtained with each brush (into four grades: poor; moderate; rich; rich with plenty of cell clusters), and to determine if one brush strongly outperformed the other (relative equivalence or strong outperformance of one brush compared with the other). A strong outperformance was defined as an obvious difference in cellular abundance between the two brushes, by comparing two slides on optical microscopic examination: almost acellular for one and very cellular for the other.

Statistical analysis

On the basis of previous unpublished pilot studies, we hypothesized a sensitivity rate of 85 % for cholangiocarcinoma with the aggressive brush (Infinity) vs. 50 % with a standard brush (RX Cytology Brush) [15]. The sample size was calculated with a type-I error of 0.05 (two-sided) and a power of 0.8: the study required a total of 50 patients.

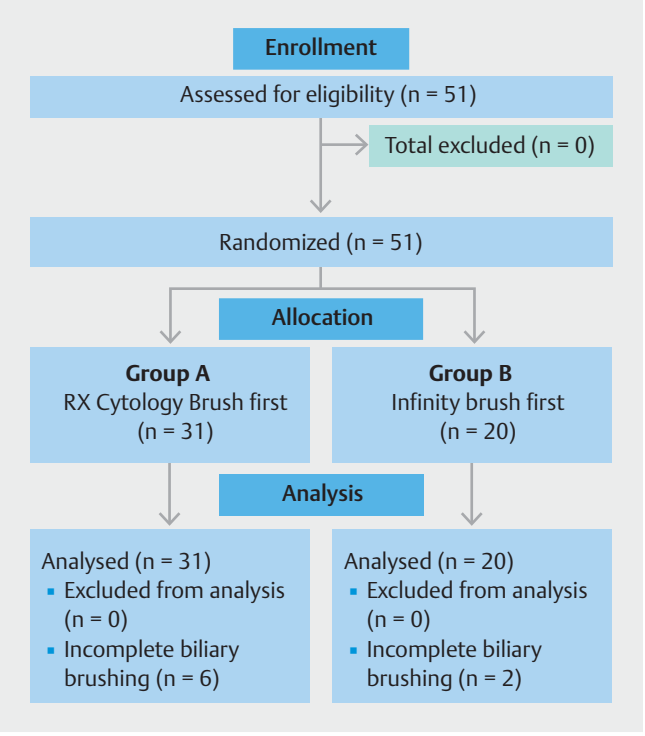
Quantitative variables were expressed as mean (SD), while qualitative variables were expressed as numbers and percentages. No sequence effect due to crossover was expected in the trial. Nevertheless, the effect of the order of use of the brushes was evaluated before each analysis. If there was a suspected order effect, the Prescott test was performed to compare paired proportions; otherwise, the McNemar's test was used.

A mixed-effects model was conducted to test for potential order and period effects. The effect of the type of brush was assessed by the McNemar's test, with the significance level set to 5 %. Statistical analyses were performed using Stata 17.0 software (StataCorp LP, College Station, Texas, USA). Negative predictive values were compared with the R DTComPair package [21].

Results

A total of 51 patients (26 men; median age 72 [interquartile range 66–80] years) were enrolled in the study and randomized (▶ **Fig. 2**).

Demographic data, stricture location data, and diagnoses are presented in ▶ **Table 1**. The location of the biliary stricture was mainly hilar (57 %), including a majority of Bismuth type III and IV strictures. Biliary brushing was feasible in all patients, but was incomplete (inability to move the brush back and forth 10 times through the stenosis owing to friction on the catheter) in two patients for the Infinity brush and in six patients for the RX Cytology Brush ($P = 0.16$). The solution used for liquid-based cytology was CytoLyt for 44/51 patients (86 %) and formalin for



▶ **Fig. 2** Study flow chart.

▶ **Table 1** Demographic data, biliary anatomic data, and final diagnosis in the 51 patients with biliary stricture without mass syndrome included in the study.

Age, median (IQR), years	**72 (66–80)**
Sex, male, n (%)	26 (51)
Symptoms, n (%)	
▪ None	4 (8)
▪ Jaundice	42 (82)
▪ Cholangitis	5 (10)
Biliary stricture location, n (%)	
▪ Common bile duct	22 (43)
▪ Liver hilum	29 (57)
▪ Type I	13 (25)
▪ Type II	1 (2)
▪ Type III	9 (18)
▪ Type IV	6 (12)
Examination before ERCP, n (%)	
▪ CT	47 (92)
▪ MRI	24 (47)
▪ EUS	38 (75)
Definitive histology, n (%)	
▪ Adenocarcinoma	43 (84)
▪ Benign	7 (14)
▪ Undetermined	1 (2)

IQR, interquartile range; ERCP, endoscopic retrograde cholangiopancreatography; CT, computed tomography; MRI, magnetic resonance imaging; EUS, endoscopic ultrasound.

the remainder. Additional sampling methods were performed during the same endoscopic procedure in most patients, including: intracholedochal biopsies under fluoroscopy in 29/51 patients (57 %), EUS-FNB in 19/51 patients (37 %), and intraductal cholangioscopy-guided biopsies in 2/51 patients (4 %). No additional sampling method was performed in 8/51 patients (16 %).

The final diagnosis was benign in seven patients and cholangiocarcinoma in 43 patients. The diagnosis of cholangiocarcinoma was obtained by: pathological analysis of biliary brushing (with at least one of the two brushes) in 36 patients (84 %); EUS-FNB (of metastasis, nodes, or the stricture if there was sufficient thickening) and/or intrabiliary biopsies performed initially or during follow-up during an additional endoscopic procedure in five patients (12 %); and from a surgical specimen in two patients (5 %). The sensitivity of intracholedochal forceps biopsy for cholangiocarcinoma was 54 % (13/24) when performed. Intracholedochal biopsies confirmed the diagnosis of cholangiocarcinoma while brushing was falsely negative in two patients. Among the seven patients (14 %) with a benign stenosis related to inflammatory cholangitis, one suffered from Crohn's disease and another from HIV.

▶ **Table 2** Comparison of demographic, biliary anatomic, and endoscopic procedural data between the two groups of patients.

	Group A[1] (n = 31)	**Group B[2] (n = 20)**	***P* value**
Age, median (IQR), years	72 (63–80)	73 (67–80)	0.76
Sex, male, n (%)	17 (55)	9 (45)	0.57
Symptoms, Yes, n (%)	3 (10)	1 (5)	> 0.99
Biliary stricture location, n (%)			0.56
▪ Common bile duct	12 (39)	10 (50)	
▪ Liver hilum	19 (61)	10 (50)	
Stenosis balloon dilation, Yes, n (%)	25 (81)	18 (90)	0.46
Morbidity, n (%)	1 (3)	2 (10)	0.55
Cholangiocarcinoma/ other, n	26/5	17/3	> 0.99

[1] Group A: RX Cytology Brush first.
[2] Group B: Infinity brush first.

One patient in the series died prematurely owing to early recurrence of jaundice and sepsis before the nature of the stenosis could be determined. The patient had an intrahepatic complex biliary stricture (Bismuth type III). Bile was initially successfully drained by two plastic stents that quickly became nonfunctional, but her general condition was deemed to have deteriorated too far to consider another ERCP.

Gastrointestinal bleeding occurred in 3/51 patients and was medically managed in each case, requiring a transfusion of four units of blood in addition to an endoscopic hemostasis procedure in one patient with sphincterotomy bleeding. No other adverse events were noted in the 51 patients, in particular there was no acute pancreatitis noted. One patient died 8 days after the ERCP from mesenteric ischemia unrelated to the biliary brushing procedure.

Primary end point

A comparison of demographic, biliary anatomic, and endoscopic procedural data between the two groups of patients is shown in ▶ **Table 2**. Results from the mixed model did not show a significant order effect ($P = 0.69$) or a significant period effect ($P = 0.17$).

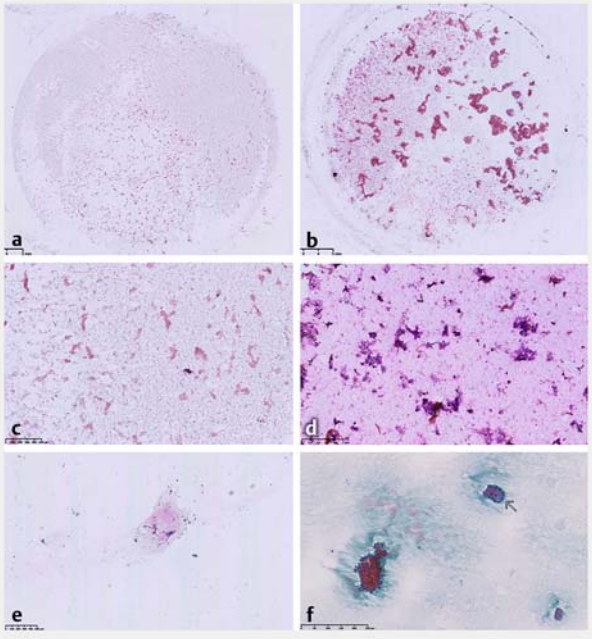
Sensitivity was 79.1 % (34/43; 95 %CI 64.0 %–90.0 %) for the Infinity brush and 67.4 % (29/43; 95 %CI 51.5 %–80.9 %) for the RX Cytology Brush ($P = 0.10$). Specificity was 100 % (95 %CI 100 %–100 %) for the Infinity brush and 100 % (95 %CI 100 %–100 %) for the RX Cytology Brush ($P > 0.99$).

Secondary end points

Results for brush cellularity (classification into four grades: poor; moderate; rich; rich with plenty of cell clusters) are reported in ▶ **Table 3**. Cellularity was significantly more often

▶ **Table 3** Comparison of cellularity obtained between the two brushes.

	Infinity brush (n = 51)	RX Cytology Brush (n = 51)	*P* value
Brush cellularity, n (%)			< 0.001
▪ Poor or moderate	20 (39)	41 (80)	
▪ Rich with or without plenty of cell clusters	31 (61)	10 (20)	
Determination of strong outperformance, in terms of cellularity obtained, n (%)			< 0.001
▪ Infinity brush > RX Cytology Brush	28 (55)		
▪ Relative equivalence	19 (37)		
▪ RX Cytology Brush > Infinity brush	4 (8)		



▶ **Fig. 3** Cytological images showing strong outperformance in terms of the cellularity obtained by: **b, d, f** the aggressive Infinity brush, compared with; **a, c, e** the standard RX Cytology Brush, on; **a, b** liquid-based cytology, at low magnification; **c, d** liquid-based cytology, at high magnification; **e, f** conventional cytological smear, with an adenocarcinoma cluster (arrow) and cell cluster shown on image **f**.

"rich" (with or without plenty of cell clusters) with the Infinity brush at 61 % (31/51) vs. 20 % (10/51) with the RX Cytology Brush ($P < 0.001$). In terms of the cellularity obtained, the Infinity brush strongly outperformed the RX Cytology Brush in 28/51 cases (55 %), while the RX Cytology Brush strongly outperformed the Infinity brush in 4/51 cases (8 %) ($P < 0.001$) (▶ **Fig. 3**). The negative predictive value was 43.8 % (7/16; 95 %CI 19.8 %–70.1 %) for the Infinity brush and 33.3 % (7/21; 95 %CI 14.6 %–57.0 %) for the RX Cytology Brush ($P = 0.10$).

Discussion

This randomized crossover study did not show any significant difference in the diagnostic accuracy for cholangiocarcinoma between the two brushes. However, the results show that the Infinity brush achieves significantly higher cellular abundance than the RX Cytology Brush.

Management of patients with biliary cholangiocarcinoma is based on surgery and/or chemotherapy [5]; however, anatomopathological diagnosis is usually required by the surgical and/or oncological teams before undertaking such treatments. Obtaining a greater abundance of cytological material, as achieved with the aggressive Infinity brush vs. the standard RX Cytology Brush in our study, usually allows for more frequent and more confident diagnoses. It would clearly seem easier for a pathologist to make a diagnosis on several adenocarcinomatous clusters than on one alone, although this study failed to support this argument.

With significantly higher cellularity, additional cytopathological examinations (such as immunohistochemistry and molecular biology for mismatch repair testing, microsatellite instability, etc.) can be performed more frequently. Such examinations are clinically relevant as they are increasingly required by the oncologist for the subsequent treatment. Moreover, the clinical relevance of a more aggressive brush for the diagnosis of cholangiocarcinoma depends on the sensitivity of the standard brush. If the sensitivity of the standard brush is 50 %, a 20 % increase should be clinically relevant (initial hypothesis of this study). Given the sensitivity for the standard brush noted in this study was 67 %, the 12 % increase with the more aggressive brush would therefore seem to be clinically relevant, but unfortunately our study does not have sufficient statistical power to make this affirmation.

Thanks to its simplicity, biliary brushing can be performed in any biliary endoscopy unit, even by nonexperienced expert endoscopists in tertiary referral centers. The sensitivity of biliary brushing is however very variable in the literature, owing to the varying patient (intrinsic or extrinsic bile duct stenosis, distal or proximal stenosis) and procedural (previous dilation, bile aspiration, standard or aggressive brush) criteria used in the studies.

In order to have a homogeneous population, and because cytopathological diagnosis is easy to obtain by EUS-FNB if there is an extrinsic stenosis, we chose to include only patients with intrinsic stenosis (i. e. without mass syndrome). As was the case in this study, intrinsic stenosis is more frequently proximal than distal [22]. As previous biliary stricture dilation [23–25] or biliary aspiration [26, 27] may optimize the sensitivity of brushing, we proposed performing these procedures during ERCP. Such optimization of the standard brushing via previous biliary stricture dilation or biliary aspiration could explain the relatively high sensitivity rate of 67 % with the standard RX Cytology Brush in this study. Given that the solution used for cytological

examination was mainly CytoLyt (86 %), we could not interpret the influence of the solution used [28].

Targeted biopsies under cholangioscopic guidance do not achieve a sensitivity for cholangiocarcinoma of more than 70 %–80 % [12], a rate that can already be obtained by aggressive biliary brushing, as was the case in this study. Recent studies have compared the sensitivity of biliary brushing and targeted biopsies under cholangioscopic guidance for cholangiocarcinoma. In a recent retrospective study of 92 patients, sensitivity was higher for cholangioscopy than for standard brushing (71 % vs. 45 %; $P=0.03$) [29]. Moreover, in the only published RCT (61 patients), the sensitivity of digital single-operator cholangioscopy-guided biopsies was significantly higher than ERCP-guided brushing, at 68.2 % vs. 21.4 %, respectively ($P<0.01$) [30]. In the latter study, the surprisingly very low standard brushing sensitivity (21.4 %) – as recognized by authors themselves – was significantly lower than this study (67 %), which was much closer to current practice. The difference between the sensitivity for cholangiocarcinoma in the two arms was also due to an unbalanced proportion of benign/malignant strictures, with nearly 50 % benign strictures in the biliary brushing arm [30]. Additionally, in the two previously mentioned studies, biliary brushing was performed with a standard brush without any optimization such as stricture dilation before brushing and/or bile aspiration. The high sensitivity for both brushes noted in our study may be a result of these optimization procedures.

Furthermore, morbidity for the standard and aggressive brushes was comparable in our study and in the previous pilot and comparative studies [15, 16]. Therefore, future trials will need to compare cholangioscopy-guided biopsies with optimized aggressive brushing with the Infinity brush in a homogeneous population of patients with biliary intrinsic strictures.

There are some drawbacks to cholangioscopy that should be noted: (i) it is technically a more challenging procedure for nonexpert endoscopists to perform; (ii) it must be planned in advance; (iii) it may be associated with higher rates of complications [31]; and (iv) it is difficult to perform routinely during an ERCP, as was the case in this study, where very few patients had a cholangioscopy as a first-line procedure, despite being treated in biliopancreatic expert units.

In addition to being cost-effective and easy to plan in an ERCP, the high sensitivity of biliary brushing for cholangiocarcinoma, as confirmed in this study, makes the procedure a possible first-line treatment in the management of bile duct stenosis. First, the brushes are safe and have a roughly equivalent price in Europe. Because the Infinity brush allows for the diagnosis of cholangiocarcinoma in approximately 80 % of patients and with more abundant cytological material than the RX Cytology Brush, this study considered the possibility of abandoning standard brushes, except for the rare cases where the rigidity of the aggressive brush does not allow for brushing. Second, ERCP with aggressive Infinity brushing is simple to plan in any biliary (even nonexpert) center and is safe, efficient, and cheap, making the procedure an attractive alternative to expensive and difficult-to-plan procedures such as targeted biopsies under cholangioscopic guidance.

This study has some limitations. First, the primary end point, as evaluated by sensitivity for cholangiocarcinoma, was higher for the aggressive Infinity brush but not significantly, probably owing to the lack of power of our study. The sample size was calculated based on very few previous published data on the aggressive Infinity brush [15]. Therefore, we obviously overestimated the sensitivity of Infinity brushing (79 % in this study vs. 85 % in pilot studies), but also underestimated standard brushing (67 % in this study [thanks to optimization procedures] vs. 50 % from the literature), leading to an insufficient study sample size.

Second, bile duct stenosis without mass syndrome is much less frequent than jaundice with pancreatic tumor mass syndrome. Low rates of patient inclusion in the study were noted in several centers, as is common in multicenter studies owing to the variable contribution of respective investigators. It is possible that not all eligible patients were invited for inclusion in the study at these centers. A large number of centers is an advantage for the reproducibility of the results, but also a weakness if the study includes too many low-inclusion centers.

Third, the abundance quantification was evaluated by a four-grade classification that we developed ourselves for the purposes of this study, because of the lack of a reference classification. This nonstandardized, but very easy-to-use, classification is a subjective semiquantitative evaluation, but the same subjectivity was applied to both brushes. Although it could be criticized for being unique to this study, the four-grade classification of cellular abundance was applied to both brushes owing to the crossover design of this study. Moreover, the outperformance of the Infinity brush compared with the RX Cytology Brush was also confirmed by the centralized blind determination of the outperformance of one brush compared with the other. In addition to seemingly higher sensitivity, cellular abundance was therefore also significantly higher with the Infinity brush.

Finally, although this study was multicenter, all patients were managed by experienced expert endoscopists in tertiary referral centers, and the centralized evaluation of specimens was interpreted by a cytopathological expert in biliary disease (A.C.). The results of this study must therefore be transposed cautiously into routine practice.

In conclusion, this randomized crossover trial shows that the Infinity brush is not significantly more effective than the RX Cytology Brush for biliary stenosis without mass syndrome in terms of sensitivity for cholangiocarcinoma diagnosis, but does allow significantly higher cellular abundance to be obtained.

Conflict of Interests

D. Karsenti has received consulting fees from Olympus. J. Privat has received consulting fees from Boston Scientific. S. Leblanc has received consulting fees from Boston Scientific and Olympus, and is on the advisory board of Alfasigma and Norgine. J. Levy has received consulting fees from Ambu. M. Schaefer has received consulting fees from Boston Scientific, lecture fees from Ferring, Alfasigma and Duomed Endoscopy, and is on the advisory board of Abbvie. G. Vanbiervliet has received consulting fees from Ambu, Boston Scientific, Cook and

Fujifilm, and lecture fees from Tillots Inc. Fujifilm, Boston Scientific and Pentax. G. Rahmi has received consulting fees from Medtronic and Fujifilm, and lecture fees from Boston Scientific and Pentax. E. Perez-Cuadrado Robles has received consulting fees from Boston Scientific. T. Wallenhorst has received consulting fees from Fujifilm and Olympus.The remaining authors declare that they have no conflict of interest.

Clinical trial

Trial Registration: ClinicalTrials.gov | Registration number (trial ID): NCT04251013 | Type of study: Prospective Multicenter Randomized Trial

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