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ORIGINAL ARTICLE

Percutaneous endobiliary forceps biopsy of biliary strictures for histopathologic examination

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PURPOSE
We aimed to investigate the feasibility, accuracy and safety of percutaneous endobiliary cholangio-forceps biopsy of biliary strictures in our institution.

METHODS
A total of 13 percutaneous transhepatic endobiliary biopsies (7 men and 6 women, mean age 66.85±16.76 years) were performed between January 2015 and March 2019 using a transluminal forceps biopsy device. Technical success, rate of complications, number of biopsy specimens, procedure and fluoroscopy time, mean radiation exposure were evaluated; sensitivity and accuracy were calculated.

RESULTS
Technical success, i.e., acquisition of at least three (median, 3.00; range, 3–5) macroscopic representative samples, could be achieved in all 13 biopsies. Access was gained via the right liver lobe in 12 of 13 cases (92.3%). All patients presented blood work indicative of cholestasis prior the intervention, with mean bilirubin 4.72±3.72 µmol/L, mean γ-glutamyl transferase 574.16 ± 360.92 IU/L, and median alkaline phosphatase 407 IU/L (165–1366 IU/L). In 12 of 13 cases (92.3%), biopsied material was sufficient for the pathologist to make a histopathologic diagnosis. Analysis revealed cases of malignancy in eight of 13 cases (61.5%), all of which turned out to be cases of cholangiocarcinoma. In four benign cases (30.8%), diagnosis was considered to be confirmed by further imaging or clinical follow-ups, which showed no signs of progressive disease. There was one case (7.7%) of a false-negative result with proof of malignancy in subsequent surgical tissue extraction. A calculation of diagnostic performance yielded a sensitivity rate of 88.9% and an accuracy rate of 92.3%. There was one case of minor and one case of major complication in our study collective, leading to an overall complication rate of 15.4%.

CONCLUSION
Percutaneous transhepatic biliary drainage (PTBD)-based forceps biopsy via the transhepatic drainage tract in patients with biliary obstruction of unknown origin is a technically feasible and safe technique with good diagnostic value rates. The procedure should be considered in patients not suitable for endoscopic strategies with indication for establishment of PTBD.

Biliary strictures may be the result of different benign and malignant alterations. Despite continuous improvement of noninvasive imaging techniques like ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI), pathologies of the biliary system often lack sufficient imaging characteristics (1, 2). In this context, benign causes like postsurgical strictures and inflammation may be associated with similar clinical and imaging characteristics as malignant conditions, of which cholangiocarcinoma is by far the most common underlying disease (3). Another demanding scenario is the appearance of restenosis after curative or palliative surgical intervention including choledochojejunostomy, since the discrimination between postoperative anastomotic fibrotic stenosis and recurrence of malignant obstruction remains challenging albeit mandatory for planning further therapy (4).

Tissue sampling with histologic and pathologic characterization of biopsy specimens represents the mainstay in the diagnostics of neoplasms (5, 6). However, yield of representative

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biopsy samples with regard to neoplasms of the biliary system remains challenging.

Percutaneous transhepatic biliary drainage (PTBD) is a well-established minimally-invasive alternative to endoscopic approaches in the treatment of obstructive jaundice (7). Since an access to the biliary system is created, PTBD also offers the option of simultaneously introducing tools for tissue sampling into the bile duct. The first report of forceps biopsy via a percutaneous access route was published in 1980 and since then this technique has been successively improved, establishing increasingly sophisticated and flexible systems (8).

To date, only a handful of studies have addressed the feasibility and safety of percutaneous endobiliary forceps biopsies (PEFB) so far, and in this context varying procedure outcomes and success rates are reported. Consequently, to address the need for more data, the purpose of this study was to investigate data for percutaneous transhepatic forceps biopsy within our institution.

Methods

Patients

In this retrospective case series, we evaluated 13 patients who underwent PEFB performed in our interventional radiology department between January 2015 and March 2019. All patients suffered from biliary obstruction. The study sample included seven men and six women with a mean age of 66.85±16.76 years ($P = 0.126$, Anderson-Darling test).

The decision for PEFB tissue sampling was based on the approval of a dedicated tumor board consisting of hepatologists, hepatic surgeons, oncologists, and interventional radiologists. In seven of 13 cases (53.8%), an endoscopic tissue sampling approach had been attempted previously, but failed due to postsurgical alterations of anatomy or tumor compression. In six of 13 cases (46.2%), PEFB was directly chosen without prior endoscopic evaluation, since it was a planned part of the therapy concept for obstructive jaundice.

Some patients had pre-existing diseases. There was a history of malignancy in six cases (46.2%) including gastric cancer in three patients (23.1%), pancreatic cancer in one patient (77%) and cholangiocarcinoma in two patients (15.4%). All these patients had already been treated with surgery and/or chemotherapy and considered tumor-free regarding the initial cancer diagnosis.

All procedures were performed by a board-certified interventional radiologist who had 21 years of experience with PTBD. All patients were examined and treated as part of routine care and gave informed consent before the intervention. The local institutional review board waived its approval (No. of approval 20190524 01).

Main points

- Percutaneous endobiliary forceps biopsy (PEFB) is a feasible technique in the diagnostic management of biliary strictures.
- In cases of a technically not achievable endoscopic strategy and planned percutaneous biliary drainage therapy, PEFB can help to avoid further invasive approaches in context of tissue sampling.
- Diagnostic value rates of PEFB-based tissue samples appear to be satisfying, especially in cases of biliary malignancy.

Preprocedural imaging

Biliary obstruction was verified prior to the intervention by using different imaging modalities: MRI in seven cases (53.6%), CT in four cases (30.8%), and US in one case (7.7%). In two cases (15.4%), prior imaging had not been performed, and indication for PEFB was based on clinical parameters. In one special case, the patient had a history of pancreatic head carcinoma that had been treated by pylorus preserving pancreaticoduodenectomy one year before. During his follow-up, this patient suffered from intermittent cholangitis and presented with persisting high levels of cholestatic parameters. In this situation, recurrent malignancy was suspected and visibility of the biliodigestive anastomosis on US was supposed to be limited. For that reason, interdisciplinary decision was made to directly and contemporaneously perform percutaneous transhepatic cholangiography (PTC) for diagnostic imaging, PEFB to obtain diagnostic biopsy specimens, and PTBD for symptomatic relief of the cholestasis.

Technique

With all patients under local anesthesia and analgosedation, all PEFB procedures were performed via a right (n=12, 92.3%) or left (n=1, 7.7%) percutaneous transhepatic approach. All procedures were carried out in our angiography suite (Siemens, Axiom Artis Zee). All patients received periprocedural antibiotic coverage. In five patients (38.5%) a PTBD had been previously positioned, in eight patients (61.5%) a percutaneous transhepatic biliary access had to be established prior to PEFB using a dedicated micropuncture set (Neff Percutaneous Access Set). In 92.3% (12/13 cases), access was created via the right liver lobe. Access was gained as described elsewhere (9). In a next step, for PTBD or Neff Set® sheath extraction, a standard hydrophilic 0.035-inch guidewire was placed through the drainage catheter or sheath beyond the major duodenal papilla or biliodigestive anastomosis, respectively. After removing the drainage/sheath, a long braided hydrophilic 7 F sheath (Flexor, Cook Medical) was inserted over the wire. After performing a cholangiogram for visualization and confirmation of the biliary stricture or stenosis as the target lesion, a flexible 5.2 F biopsy forceps (Cook Medical) was inserted via the hemostasis valve of the sheath. The biopsy forceps was expelled into the sheath and advanced to the target level. Exposure of the biopsy forceps was achieved by withdrawing the sheath over the wire. Using the "cross and push" technique, jaws of the forceps were pressed against the target lesion in an opened position. After that, the jaws were closed and the tissue specimen was cut off by retrieving the forceps into the sheath. After removal of the biopsy forceps from the sheath the biopsy specimen was secured. The tissue biopsies were defined as being suitable for histopathologic examination if a solid specimen with a length of at least 1 to 2 mm had been obtained. The tissue core biopsies were fixed in a formalin solution for histopathologic examination. PEFB procedures were concluded by exchange of the sheath for a PTBD catheter over the wire, its fixation, and angiographic documentation (Figs. 1, 2).

Outcome measures

Primary outcome measures of the study were the technical success rate and the rate of complications. In addition, the mean number of biopsy specimens, the mean total procedure time (from start until a suitable biopsy sample is obtained), the mean fluoroscopy time, and the mean radiation exposure in mSv were evaluated. In this context, PEFB was defined as successful if representative tissue for a definitive histopathologic diagnosis was obtained and agreed with the final diagnosis. All malignant diagnoses were categorized as representative. Benign diagnoses were classified as representative if a benign neoplasm or

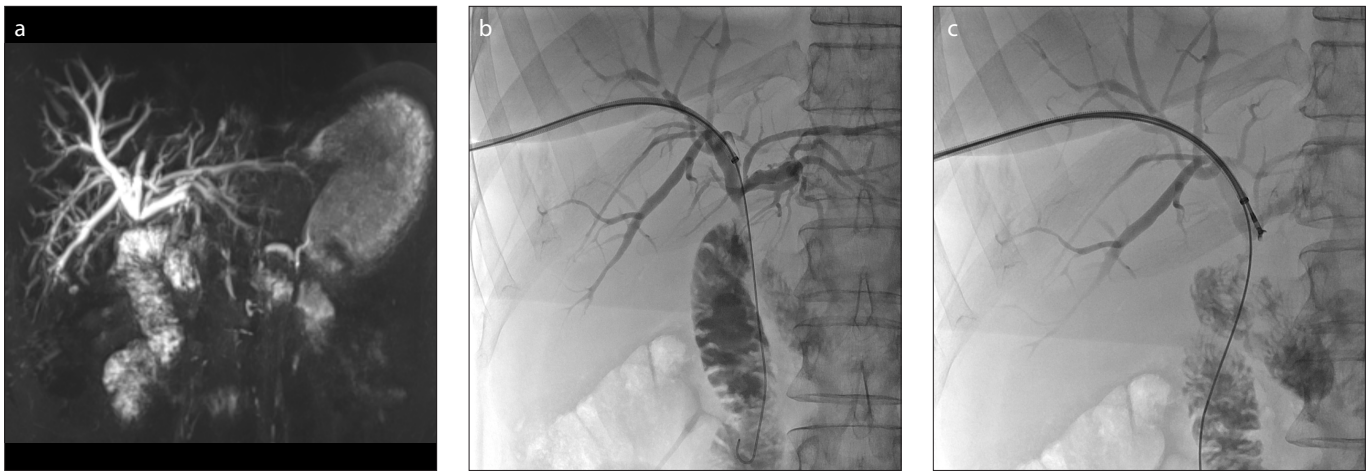

Figure 1. a–c. A 56-year-old female patient with history of pancreatic head carcinoma and Whipple procedure presented with clinical signs of increasing cholestasis confirmed by MRI and ultrasound imaging. In panel **(a)**, corresponding MRI examination with MRCP (MIP reconstruction) prior the intervention revealed intrahepatic cholestasis with no signs of recurrent malignancy. Panel **(b)** shows PTBD previously positioned via the right hepatic lobe. The corresponding percutaneous transhepatic cholangiogram revealed a stenosis in the area of the biliodigestive anastomosis. Panel **(c)** shows insertion of the biopsy forceps through a sheath with an opened position of the jaws for tissue sampling within the stricture. Histology revealed scar tissue without evidence of malignancy.

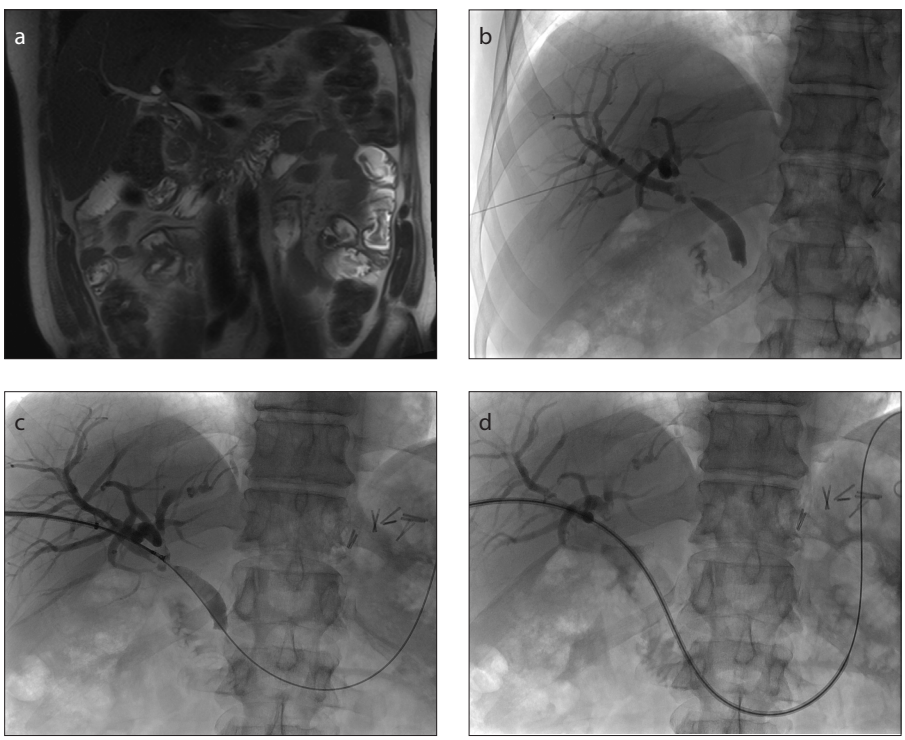

Figure 2. a–d. A 43-year-old male patient with esophageal cancer and oncologic transhiatal gastrectomy. In panel **(a)**, MRI examination performed prior the intervention reveals progressive cholestasis in the T2 HASTE sequence with caliber irregularity and stenosis proximal to the confluence of the right and left intrahepatic bile duct. In panel **(b)**, following percutaneous transhepatic puncture of a peripheral bile duct in the right lobe, PTB shows nodge of contrast agent in the biliary bifurcation due to a significant stenosis of the common bile duct. In panel **(c)**, after establishing a 7 F sheath, the biopsy forceps was introduced into the lesion and four tissue specimens were sampled for histology. Finally, in panel **(d)**, a 8.5 F drainage catheter was placed over a wire for internal and external drainage. Histology revealed inflammation and no malignancy.

specific infection was diagnosed and as nonrepresentative if the biopsy sample yielded nonspecific benign changes (e.g., inflammation or fragments of fibrosis). Non-diagnostic specimens (e.g., scanty tissue or blood) were also considered as nonrepresentative. All nonrepresentative tissue samples were verified by repeated PEFB procedures, open surgical biopsy or 6 to 12 weeks of follow-up using cross-sectional imaging examinations like US, CT, or MRI. According to this categorization, measures of diagnostic performance (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV], and accuracy) were calculated.

Definition of complications were based on SIR reporting standards concerning percutaneous transhepatic biliary interventions (10): minor complications included those resulting in no therapy and no consequence (class A) or minimal therapy and no consequence including overnight admission for observation only (class B). Major complications included those that required therapy or minor hospitalization for less than 48 hours (class C); those that required major therapy, unplanned increase in level of care, or prolonged hospitalization for more than 48 hours (class D); those that resulted in permanent adverse sequelae (class E); and those that resulted in death (class F).

Follow-up

Imaging follow-up included CT in seven cases (53.8%), MRI in two cases (15.4%), US in one case (7.7%) and PTC via existing percutaneous drainage catheter in two cases (15.4%). In one case (7.7%), no further follow-up was performed in our institution.

Statistical analysis

Descriptive data were presented as mean±SD (normal distributed variables) or

median with range (non-normalized variables), as appropriate; categoric data were presented as number and percentage. With regard to assessment of normality, the Anderson-Darling test was used, rejecting the hypothesis of normality when the *P* value is less or equal to 0.05. Sensitivity, specificity, PPV, NPV, and accuracy were given as measures of diagnostic performance. Statistical analysis and the evaluation of the data were performed with specialized computer algorithm (Microsoft Excel).

Results

In all 13 PEFB interventions, correct position of biopsy device and acquisition of at least three representative samples could be achieved, leading to a technical success rate of 100%. The number of samples ranged between 3 and 5, with a median of 3 ($P < 0.001$, Anderson-Darling test). In all cases, relevant stenosis/stricture could be identified by cholangiography. Location of stenosis and site of sample extraction was biliary bifurcation in four cases, common bile duct in five cases, biliodigestive anastomosis in three cases and intrahepatic ductus hepaticus sinister in one case.

Mean procedure time was 21.0±5.7 min ($P = 0.712$, Anderson-Darling test). Mean radiation exposure time was 7.08±1.89 min ($P = 0.295$, Anderson-Darling test) and radiation exposure resulted in mean measurements of 122.91±69.13 mSv ($P = 0.746$, Anderson-Darling test).

In all patients, laboratory analysis revealed elevated cholestatic parameter, with a mean bilirubin of 4.72±3.72 µmol/L ($P = 0.284$, Anderson-Darling test), mean γ-glutamyl transferase (GGT) of 574.16±360.92 IU/L ($P = 0.159$, Anderson-Darling test), and median alkaline phosphatase (AP) of 407 IU/L (165–1366 IU/L) ($P = 0.045$, Anderson-Darling test).

In 12 of 13 cases (92.3%), biopsied material was sufficient for the histopathologist to render a final diagnosis. In one case (7.7%), histology indicated adenocarcinoma, but histologic result was not considered as certain, as provided tissue sample was too small and yielded crush artifacts. However, a subsequently performed explorative laparoscopy and surgical biopsy confirmed the diagnosis of a progressive metastatic biliary adenocarcinoma. Therefore, the result was evaluated as true-positive.

In 13 biopsy procedures, eight lesions (61.5%) were malignant and five lesions (38.5%) were benign. In all cases of malignancy, final diagnosis was cholangiocarcinoma (n=8, 100%). In two cases (25%) of diagnosed malignancy, diagnosis was additionally confirmed by further open surgical biopsy. Final diagnosis in the benign samples included inflammation (n=3, 60%), scar tissue within the biliodigestive anastomosis (n=1, 20%) and secondary sclerosing cholangitis (n=1, 20%). In four cases (30.8%) of nonrepresentative tissue samples further follow-up imaging did not show any increase of lesion size or other evidence for malignancy (contrast enhancement). Eight PEFB samples (61.5%) revealed a true-positive, four biopsies (30.8%) a true-negative finding, and one biopsy (7.7%) showed a false-negative result. There were no false-positive findings in our study sample. The overall diagnostic accuracy rate obtained for PEFBs was 92.3%. The overall sensitivity was 88.9%. The NPV and PPV for PEFBs were 80% and 100%, respectively.

The overall complication rate was 15.4% (2/13 cases) with one case of minor (7.7%) and one case of major complication (7.7%) in our study. Minor complication occurred as postprocedural cholangitis, which was successfully treated with antibiotics with no further need of therapy. In the other case, subcapsular hematoma led to initial drop of hemoglobin requiring transfusion of two units of red blood cell concentrates as well as overnight observation in an intensive care unit. The hematoma necessitated no further surgical or interventional treatment and showed autonomous regression which was confirmed by multiple CT follow-ups. Results are summarized in the Table.

Discussion

Nowadays, tissue sampling for cytologic and histologic characterization of tumor cells as well as the identification of special subtypes based on immunohistochemistry constitutes an important part of precise diagnostic management of neoplasms, as the results determine future individual treatment protocols (5, 6). Different tissue sampling techniques have been used concerning biliary strictures with reported high variation of sensitivity and accuracy (11, 12). Although modern percutaneous puncture techniques guided by CT or US enable safe and sensitive tissue samplings of almost every anatomic area, they revealed poor sensitivity and specificity rates as far as neoplasms of the biliary system are concerned and are often limited in distinguishing between benign and malignant biliary strictures (3).

Since most biliary neoplasms have their origin in biliary epithelium, any dedicated endoluminal approach seems to be promising for the acquisition of biopsy specimens. Based on the hypothesis that tumor cells of the biliary system are constantly excreted into bile, cytologic examination of bile collected via a percutaneous or endoscopic technique offers a low-invasive opportunity of cytodiagnosis. However, this method seems to be limited by the diagnostic yield in terms of sensitivity (13, 14).

Endoscopy-based techniques are more reliable, with high rates of accuracy and even improved diagnostic yield when combined (15). They include endoscopic US with fine-needle aspiration (EUS-FNA), which represents the gold standard, and endoscopic retrograde cholangiopancreatography (ERCP) with biliary brushing or forceps biopsy (16, 17). EUS-FNA has been demonstrated to be superior to other ERCP-guided techniques, although tumor seeding along the needle tract seems to be a major concern in some cases (17, 18). Nevertheless, tissue sampling guided by an endoscopic retrograde cholangiography approach is sometimes not feasible due to altered anatomy after oncological surgery and biliodigestive anastomosis (19). In addition, ERCP-guided biopsies in proximal parts of the biliary system often lack satisfying accuracy rates and have their limitations for more proximal hilar lesions (17). Furthermore, in addition to technical difficulties, the appearance of a new stricture may be the result of cicatricial stenosis or tumor recurrence, respectively, and is difficult to differentiate based on imaging findings or laboratory diagnosis alone (4).

PTBD is the treatment of choice in cases of obstructive jaundice, when ERPC-based techniques fail. Moreover, it commonly presents a well-established strategy in the preoperative or palliative therapy of biliary obstruction (6). In this context, PEFB procedures seem to be a reasonable alternative to other tissue sampling techniques, since it can be easily conducted using an already created PTBD tract. Consequently, in case of an established PTBD the need for a further invasive procedure like EUS-FNS should be carefully considered. As a matter of fact, although often hardly visible in imaging modalities like CT or MRI, neoplasms of cholangiocellular origin frequently lead to apparent stenosis or contrast medium notches in percutaneous cholangiogra-

Table. Clinical and laboratory characteristics of the study population	
Number of patients	13
Age (years), mean±SD	66.85±16.76
Male:female ratio	1.2:1
Pre-existing malignancy, n/N (%)	6/13 (46.2)
Gastric cancer	3/13 (23.1)
Pancreatic cancer	1/13 (7.7)
Cholangiocarcinoma	2/13 (15.4)
Cholestasis parameters before the procedure	
Bilirubin (µmol/L), mean±SD	4.72±3.72
GGT (IU/L), mean±SD	574.16±360.92
AP (IU/L), median (IQR)	407.00 (165–1366)
Approach side right:left ratio	13:1
Biopsy location, n/N (%)	
Common bile duct	5/13 (38.5)
Bifurcation of bile duct	4/13 (30.8)
Intrahepatic bile duct	1/13 (7.7)
Biliodigestive anastomosis	3/13 (23.1)
Number of samples, median (IQR)	3.00 (3–5)
Fluoroscopy time (min), mean±SD	7.08±1.89
Radiation exposure (mSv), mean±SD	122.91±69.13
Technical success, n/N (%)	13/13 (100)
Sufficiency rate, n/N (%)	12/13 (92.3)
Histologic diagnosis, n/N (%)	
Benign	5/13 (38.5)
Inflammation	3/13 (23.1)
Secondary sclerosing cholangitis	1/13 (7.7)
Cicatricial tissue without inflammation	1/13 (7.7)
Malignant	8/13 (61.5)
Cholangiocarcinoma	8/13 (61.5)
Sensitivity, %	88.89
Specificity, %	100.00
PPV, %	100.00
NPV, %	80.00
True-positive, n/N (%)	8/13 (61.5)
True-negative, n/N (%)	4/13 (30.8)
False-positive, n/N (%)	0/13 (0)
False-negative, n/N (%)	1/13 (7.7)
Complications, n/N (%)	2/13 (15.4)
Major	1/13 (7.7)
Minor	1/13 (7.7)

SD, standard deviation; GGT, γ-glutamyl transferase; AP, alkaline phosphatase; IQR, interquartile range; PPV, positive predictive value; NPV, negative predictive value.

phy, which may simplify tumor localization with subsequent biopsy acquisition (12). In accordance with other studies, our experience relating to the feasibility of this technique seems to be satisfactory with a technical success rate of 100%.

Brush cytology as another PTBD-based approach usually reveals lower rates of sensitivity and specificity compared with forceps biopsy especially in diagnosis of cholangiocarcinoma, which seems to be reasonable because of the more superficial nature of tissue sampling in this technique (13, 20). One study describes higher sensitivity rates when forceps biopsy and brush cytology are combined suggesting both as a tandem approach (21).

Restricted biopsy depth on the other hand also leads to limitations of PEFB concerning diagnosis of extrabiliary neoplasms associated with biliary obstruction due to lymphonodal metastasis, tumor infiltration or compression of the biliary system (22). In our series, one false-negative result (7.7%) was represented by a case of gastric cancer causing extrinsic constriction of ductus hepaticus communis. In this case, the histopathologic result was based on the tissue sampling using PEFB and indicated inflammation, whereas explorative laparotomy and biopsy performed shortly thereafter yielded malignant diagnosis. Because of the limited tissue acquisition from intraluminal lesions, this approach might be considered as non-representative in cases of extrabiliary tumor manifestation that have not infiltrated the biliary duct walls. This assumption seems to be in accordance with other studies (12, 22). Published sensitivity rates were shown to be significantly higher in patients with cholangiocarcinoma than in patients with other underlying malignancies (12). In our series, all patients with confirmed malignancy had cholangiocellular adenocarcinoma based on percutaneous sample acquisitions.

With a low number of false negative cases, our results show high correlation with procedure outcomes of other studies, emphasizing the role of percutaneous transhepatic forceps biopsy in exclusion of malignancy (12, 13, 23, 24). Our sensitivity rate of 88.9% and accuracy rate of 92.3% are comparable with results of other current studies (13, 25, 26).

Nevertheless, some studies reported lower sensitivity rates in the detection of malignancy (20). As suggested by Fohlen et al. (24), one factor influencing sensitivity values might be the location of biliary strictures and thus the site of biopsy. Accordingly, specimen acquisition in upper parts of the biliary system like the biliary bifurcation or intrahepatic bile duct showed higher sensitivity rates than in more extrahepatic parts (12, 24). Since hilar lesions revealed significant lower

sensitivity rates when endoscopic biopsy was performed, PEFB should be considered as potential first-line approach in strictures of the upper biliary system distant from the papilla (24–26). Primary origin of tumor causing biliary obstruction seems to be another factor affecting sensitivity. Again, due to the limited depth of biopsy, metastatic disease without infiltration of biliary system may be often associated with significantly more false negative results (12, 22, 25). The absence of false positive results in our small sample of patients as well as in other studies indicates a high diagnostic value in case of malignant result leading to a PPV of 100% (13).

A potential disadvantage of the percutaneous endobiliary forceps biopsy approach may be the occurrence of "crush artifacts" caused by the destruction of tissue samplings due to the biopsy maneuver per se and, resulting in a nondiagnostic (nonrepresentative) tissue sample (26). In our study, there was one case of uncertain histopathological result (7.7%) due to the degraded quantity as well as quality of specimen.

Reported complications in the literature associated with PEFB involve significant bleeding or perforation of the biliary tract at the biopsy site (12). The occurrence of subcapsular hematoma in our study had to be attributed to a *de novo* tract creation with an increased risk of capsular injury in comparison to a PTBD tract that had already been established days or weeks before. Cholangitis, which was a minor procedure-related complication in our study group, has to be considered as primarily caused by the percutaneous approach and not by forceps biopsy itself. In general, distinguishing between complications attributed to the drainage or access approach or to the acquisition of tissue remains impossible in some cases. With a total complication rate of 15.4%, our experiences concur with those of other studies. However, it proved to be higher than the rate of the largest study to date, conducted by Jung et al. (12, 25, 27).

This study has its limitations. The study design is retrospective in nature and the small number of patients constitutes some limitation of statistical validity. Further investigations with larger patient numbers, preferable in a multicenter study design, would allow more detailed findings, defining the value of this technique more precisely. In this study, we predominantly analyzed patients who were not suitable for endoscopic strategies. The implementation of a control group with comparison of study outcomes between PTBD- and ERCP-based tissue acquisition would be of great interest. In this setting, a standardized imaging algorithm before and after the intervention as a predefined follow-up, would create stable study framework conditions and thus improve comparability.

In conclusion, PEFB is a feasible procedure in the diagnostic management of biliary strictures with high rates of sensitivity and accuracy and a moderate number of complications. This technique should be considered in cases of failed endoscopic approaches as well as in cases where PTBD is contemporaneously planned or indicated.

Conflict of interest disclosure

The authors declared no conflicts of interest.

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