



## Endoscopic transpapillary brush cytology and forceps biopsy in patients with hilar cholangiocarcinoma

Andreas Weber, Claus von Weyhern, Falko Fend, Jochen Schneider, Bruno Neu, Alexander Meining, Hans Weidenbach, Roland M Schmid, Christian Prinz

Andreas Weber, Jochen Schneider, Bruno Neu, Alexander Meining, Hans Weidenbach, Roland M Schmid, Christian Prinz, Department of Internal Medicine II, Technical University of Munich, Munich 81675, Germany

Claus von Weyhern, Falko Fend, Department of Pathology, Technical University of Munich, Munich 81675, Germany

**Author contributions:** Weber A, Weidenbach H, Schmid RM, and Prinz C designed research; Schneider J, Neu B, Meining A, von Weyhern C, and Fend F performed research; Weber A, Schneider J, Weidenbach H and Prinz C analyzed data; Weber A, Schmid RM, von Weyhern C, and Prinz C wrote the paper.

**Correspondence to:** Professor Christian Prinz, Department of Internal Medicine II, Technical University of Munich, Ismaningerstrasse 22, München 81675, Germany. [christian.prinz@lrz.tum.de](mailto:christian.prinz@lrz.tum.de)

Telephone: +49-89-41405973 Fax: +49-89-41407366  
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### Abstract

**AIM:** To evaluate the sensitivity of brush cytology and forceps biopsy in a homogeneous patient group with hilar cholangiocarcinoma.

**METHODS:** Brush cytology and forceps biopsy were routinely performed in patients with suspected malignant biliary strictures. Fifty-eight consecutive patients undergoing endoscopic retrograde cholangiopancreatography (ERCP) including forceps biopsy and brush cytology in patients with hilar cholangiocarcinoma between 1995-2005.

**RESULTS:** Positive results for malignancy were obtained in 24/58 patients (41.4%) by brush cytology and in 31/58 patients (53.4%) by forceps biopsy. The combination of both techniques brush cytology and forceps biopsy resulted only in a minor increase in diagnostic sensitivity to 60.3% (35/58 patients). In 20/58 patients (34.5%), diagnosis were obtained by both positive cytology and positive histology, in 11/58 (19%) by positive histology (negative cytology) and only 4/58 patients (6.9%) were confirmed by positive cytology (negative histology).

**CONCLUSION:** Brush cytology and forceps biopsy have only limited sensitivity for the diagnosis of malignant hilar tumors. In our eyes, additional diagnostic techniques should be evaluated and should become routine in patients with negative cytological and histological findings.

### INTRODUCTION

Current epidemiological data analysis reveals an increasing incidence and mortality from cholangiocarcinoma<sup>[1-4]</sup>. Cholangiocarcinomas are topographically categorized as intrahepatic or extrahepatic carcinomas. Extrahepatic cholangiocarcinomas are further subdivided into hilar, medial and distal carcinomas. The most common type are hilar cholangiocarcinomas which are classified into 4 stages according to the bismuth classification: stage I or II for tumors expanding up the hilus, type III A/B infiltrating right or left hepatic duct, and stage IV with infiltration of both hepatic ducts and sub segments<sup>[5]</sup>. Because of late presentation of symptoms and the difficult distinction between benign and malignant strictures the prognosis of patients with hilar cholangiocarcinoma is poor, and survival data for advanced stages reported so far show a very limited life expectancy<sup>[6-8]</sup>. Surgery is the only curative treatment in patients with cholangiocarcinoma. The results are more favourable for patients with early-stage disease. Therefore, a reliable diagnostic procedure is of great importance for patient's chance for survival. Cholangiocarcinomas are diagnosed by a combination of imaging techniques and endoscopic procedures, including endoscopic retrograde cholangiography and/or percutaneous transhepatic cholangiography<sup>[9-13]</sup>. Cholangiocarcinomas often grow longitudinally along the bile duct rather than in radial direction

away from the bile duct. Consequently, imaging techniques including ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) are of limited sensitivity for the detection of the malignant lesion<sup>[14-17]</sup>.

Biliary tissue collection during endoscopic procedures is widely used for distinction between benign and malignant strictures and provides the only definitive diagnosis that can be used for establishing therapeutic strategies. To obtain tissue samples, brush cytology and/or forceps biopsy were routinely performed in patients with suspected malignant biliary strictures. Several studies have evaluated the cancer detection rate of brush cytology since the first description by Osnes *et al*<sup>[18]</sup> in 1975. Brush cytology is the most common tissue sampling technique and can be performed for most biliary strictures detected by endoscopic retrograde cholangiography. It is generally safe, requires little time, and is technically easier compared to forceps biopsy. The sensitivity of brush cytology for diagnosis of malignant biliary strictures ranges from 30% to 60% in most published series<sup>[19-21]</sup>. Tissue samples for histological investigation can be obtained from biliary strictures by using forceps. This technique is more time consuming than brushing and is less widely used, but it provides a sample of subepithelial stroma. Consequently, only histological investigation allows diagnosing invasive growth. In patients with malignant biliary stricture the overall cancer detection rate of forceps biopsy is higher compared to brush cytology, ranging from 43% to 81%<sup>[22-24]</sup>.

To date, no study exists investigating the sensitivity of these techniques in patients with hilar cholangiocarcinoma (Klatskin tumors). The present study was designed to address the question of diagnostic sensitivity for endoscopic transpapillary brush cytology and forceps biopsy in a homogeneous group of 58 patients with hilar cholangiocarcinoma.

## MATERIALS AND METHODS

The study included 58 consecutive patients (31 male, 27 female, median age  $68 \pm 10.6$  years) with malignant hilar bile duct tumors who were treated from 1995 to 2005 in the II. Medizinische Klinik, Klinikum rechts der Isar at the Technical University Munich. The biliary stricture localization was classified in relation to the confluence of the hepatic ducts as described by Bismuth<sup>[5]</sup>. Bismuth stage was classified by endoscopic retrograde cholangiography at the time of primary diagnosis.

Patients included into this study were identified by using an endoscopic database. The analysis of our database was performed as follows: First, all patients with bile duct strictures diagnosed in the period from 1995 to 2005 were identified. Subsequently, cholangiograms of these patients were reviewed and the bile ducts strictures were divided according to its localization. In the next step we tried to clarify the genesis of the hilar stricture by analysing patient records including follow up. In total, 96 patients with hilar cholangiocarcinoma could be identified. In 58 out of 96 patients forceps biopsy and brush cytology was performed.

All of the following inclusion criteria had to be confirmed: (I) Diagnosis of hilar cholangiocarcinoma (histo-

logically/cytologically positive for malignancy or patients for whom the subsequent clinical course confirmed malignancy); (II) Transpapillary brush cytology and forceps biopsy as diagnostic approach. Exclusion criteria were as following: (1) Intrahepatic cholangiocarcinoma; (2) Distal cholangiocarcinoma; (3) Biliary obstruction due to liver metastasis; (4) Hepatocellular carcinoma; (5) No tumor progression in patients with negative histology/cytology; (6) Survival time more than 18 mo in patients with negative histological/cytological investigation. The exclusion criteria were scheduled in order to achieve a homogeneous patients group with hilar cholangiocarcinoma without tumors of different origin.

In all patients, routine diagnostic procedures included abdominal ultrasound, abdominal CT scans and endoscopic retrograde cholangiopancreatography (ERCP) with tissue sampling. After reception of the histology results, patients with strictures were associated with tumors located in the hilar region, respectability was primarily discussed with the abdominal surgeon. In 28 selected patients cholangioscopy was performed. Overall 16 out of 58 patients were transferred to the surgery unit and operation was performed. In patients with negative histology/cytology after first attempt of forceps biopsy/brush cytology another diagnostic approaches including percutaneous ultrasound guided fine needle biopsy, endoscopic ultrasound guided fine needle biopsy, or percutaneous transhepatic cholangioscopic guided biopsy was performed. In patients with furthermore unclear diagnosis, another attempt of transpapillary tissue sampling was performed after 3 mo.

Endoscopic retrograde cholangiography (ERC) was performed with a standard videoduodenoscope Olympus TFJ 160-R (Olympus, Hamburg, Germany). The first ERC comprised an endoscopic sphincterotomy (EPT) which was performed using an Olympus papillotome (Olympus, Hamburg, Germany) introduced over a Terumo guide wire. Under radiographic guidance using contrast fluid bile duct strictures were localized. The forceps was advanced as far as possible into the stricture in the closest position, then forceps was opened and the specimen was obtained. If the forceps could not enter the stricture, the open forceps was gently pushed against the distal end of the bile duct stricture. The specimen was obtained by closing the forceps in this position and fixed in 4% formalin. Bile duct strictures were brushed with multiple, rapid, to and fro movements using a standard 6 French (F) Geenen spring tip sheathed cytology brush inserted over a standard guide wire. The cellular material adherent to the brush was directly transferred to a glass slide in the endoscopy room. The brush was subsequently stored in 50% ethanol and processed at the Institute of Pathology to collect the remaining material. The specimens were evaluated by an experienced cytopathologist. Cytological results were recorded as positive or negative for malignant cells. According to the usual cytologic and histologic classification system, in the current study suspicious and positive specimens were considered as positive, whereas negative specimens and unspecific reactive changes were classified as negative.

In two patients, passage of the Y-shaped stenosis of

**Table 1** Patients characteristics, physical and laboratory parameters on admission

	Patient characteristics	Standart values	Scale unit
Number of patients	58	-	-
Gender	Male (m): 31 (53.4%) Female (f): 27 (46.6%)	-	-
Bismuth II	7	-	-
Bismuth III	25	-	-
Bismuth IV	26	-	-
Bilirubin	6.9 ± 7.6	< 1.2	mg/dL
Alkaline phosphatase	559 ± 270	40-120	U/L
γ-GT	404 ± 362	< 66	U/L
Leucocytes	8.8 ± 3.8	4-9	G/L

**Table 2** Number of tissue sampling sessions

Number of tissue sampling sessions	Number of patients	Number of samples
1 session	47	47
2 sessions	8	16
3 sessions	2	6
4 sessions	1	4
	58	73

a complex hilar tumor did not allow a stable passage of a wire guided brush into the periphery after dilatation or use of an internal bougie. Since the tip of the brush runs parallel to the wire although guided by a small plastic shaft, the brush itself is not adherent to the wire (Boston Scientific, Boston, USA). Therefore, brush cytology could only obtained in these two patients by positioning of the brush in the tumor area, moving backward and forward in the tumor area with the tip, although a complete passage with the brush over the tumor area could not be achieved due to a rectangular path of the bile duct within the tumor. Both patients were positive for malignancy. In 4 patients material received by forceps biopsy was not suitable for diagnosis. In these patients another endoscopic procedure was performed.

## RESULTS

### Patient's characteristics

Patients entered into this study had the following characteristics: mean age 68 ± 10.6 years, bilirubin level 6.9 ± 7.6 mg/dL, alkaline phosphatase 559 ± 270 U/L, γ-GT 404 ± 362 U/L, and leucocytes 8.8 ± 3.8 G/L. At the time of their initial diagnosis 7/58 patients were related to bismuth stage II, 25/58 to bismuth stage III, and 26/58 to bismuth stage IV (Table 1). The final diagnosis was made by surgical specimens (*n* = 3), autopsy (*n* = 1), percutaneous ultrasound guided fine needle biopsy (*n* = 3), endoscopic ultrasound guided fine needle biopsy (*n* = 3), endoscopic transpapillary forceps biopsy and brush cytology (*n* = 35), and percutaneous transhepatic cholangioscopic guided biopsy (*n* = 4). In 9 patients, final diagnosis could not be confirmed by histological or cytological diagnostic methods. Therefore, diagnosis was substantiated by clinical course and survival time. Each of these 9 patients has

**Table 3** Sensitivity of brush cytology and forceps biopsy in patients with hilar cholangiocarcinoma *n* (%)

	Positive for malignancy
Brush cytology	24/73 <sup>1</sup> (32.9) 24/58 <sup>2</sup> (41.4)
Forceps biopsy	31/73 <sup>1</sup> (42.5) 31/58 <sup>2</sup> (53.4)
Combination of cytology and biopsy	35/73 <sup>1</sup> (47.9) 35/58 <sup>2</sup> (60.3)

<sup>1</sup>Samples; <sup>2</sup>Patients.

a limited survival time ranging from 4 to 8 mo. Cholangioscopy was performed in 28 out of 58 patients. 10/29 patients had an infiltrative growth and 18/28 patients had a nodular growth.

### Number of tissue sampling sessions

In case of negative results for malignancy, brush cytology and forceps biopsy was repeated. The mean number of tissue sampling sessions was 1.3 per patient. Table 2 gives an overview of the number of tissue sampling sessions. One patient of our series had 4 histological/cytological investigations. In this patient, all histological/cytological samples were negative and definite diagnosis was obtained by autopsy.

### Sensitivity of endoscopic transpapillary brush cytology

Brush cytology was performed in 58 patients. Positive results for malignancy were obtained in 24/58 patients. Overall, brushing was performed 73 times in 58 patients, and malignancy was obtained in 24 of 73 cytological samples. The overall sensitivity of brush cytology for diagnosis of hilar cholangiocarcinoma was 41.4% regarding number of patients, and 32.9% regarding number of samples, respectively (Table 3).

### Sensitivity of endoscopic transpapillary forceps biopsy

Forceps biopsy was performed in 58 patients. Malignancy was detected in 31 of 58 patients. If biopsy specimens were diagnosed as non-malignant, forceps biopsy was repeated up to 4 times. Overall forceps biopsy was performed 73 times in 58 patients, and was positive for malignancy in 31 of 73 histological tissue samples. Consequently, the sensitivity of forceps biopsy for the diagnosis of hilar cholangiocarcinoma was 53.4% regarding number of patients, and 42.5% regarding number of tissue samples, respectively (Table 3).

### Sensitivity of brush cytology and forceps biopsy in a combined approach

The combination of the results of brush cytology and forceps biopsy resulted in a minor increase in diagnostic sensitivity to 60.3% (35/58 patients). Table 4 shows the distributions of positive and negative results for the 58 patients. 34.4% (20/58 patients) of diagnosis were obtained by both positive cytology and positive histology, 19% (11/58 patients) by positive histology (negative cytology)

**Table 4** True positive and false negative results for cytological and histological investigation

	Histology positive		Histology negative		Total	
	Samples	Patients	Samples	Patients	Samples	Patients
Cytology positive	20	20	4	4	24	24
Cytology negative	11	11	38	23	49	34
Total	31	31	42	27	73	58

and only 6.9% (4/58) were confirmed by positive cytology (negative histology). Table 4 illustrates the distribution of positive and negative results of cytological and histological investigations in 73 samples.

## DISCUSSION

Suspicious biliary strictures are a diagnostic challenge in endoscopic practice. Tissue collection during endoscopic retrograde cholangiography (ERC) and/or percutaneous transhepatic biliary drainage (PTBD) are the most common techniques for providing a definitive diagnosis<sup>[25]</sup>. The current study intended to determine the diagnostic value of transpapillary forceps biopsy and brush cytology in patients with hilar cholangiocarcinoma. Pugliese *et al*<sup>[23]</sup> reported sensitivity of both brush cytology and forceps biopsy in 36 patients with malignant bile duct stricture, but did not focus on hilar lesions. The patients in this study had a wide spectrum of diagnosis including pancreatic cancer (12/36), cholangiocarcinoma (10/36), intra-ampullary carcinoma (9/36), metastatic cancer (3/36), and malignant islet cell tumor (2/36). Cytological and histological investigation was positive in 19/36 patients. This indicates that the overall sensitivities of both techniques were almost identical (53%). In 6/10 patients (60%) with cholangiocarcinoma histology was positive, whereas cytology was positive in 7/10 patients (70%). Details regarding localization of the cholangiocarcinoma were not given in this paper. Similar to these data, Ponchon *et al*<sup>[22]</sup> also investigated a heterogeneous patient group with malignant bile duct strictures. In this series, 27/73 patients had cholangiocarcinomas, however also without specification of stricture localization. In 12/25 patients (48%) cytology, in 7/16 patients (44%) biopsy was positive for malignancy. The combination of both cytology and biopsy increased the sensitivity in 14 patients with cholangiocarcinoma to 86%.

Our study comes to the conclusion that diagnostic sensitivity is poor, since only 60.3% of diagnoses were made correctly using brush cytology and forceps biopsy in combined approaches. In our series, sensitivity of transpapillary brush cytology was 32.9% regarding number of cytological samples, and 41.4% regarding number of patients; whereas sensitivity of forceps biopsy was 42.5%, and 53.4%, respectively.

Endoscopic ultrasonography (EUS) complements the role of endoscopic and percutaneous transhepatic approaches and may provide a tissue diagnosis through fine-needle aspiration (FNA). Recently, the yield of EUS-FNA in patients with suspected cholangiocarcinoma was evaluated<sup>[26]</sup>. The authors reported a diagnostic sensitivity of 86%.

However, another group reported lower rates of diagnostic sensitivity (45%) for detection of bile duct lesions by using ultrasound guided fine needle aspiration<sup>[27]</sup>. Thus, EUS-FNA may represent an alternative approach in the diagnosis of cholangiocarcinoma, especially in patients with negative brush cytology and forceps biopsy findings. One of the major limitations of endoscopic brush cytology from bile duct strictures is the poor quality of cytologic samples.

Recently, investigators have attempted to improve diagnostic assessment with an advanced cytological technique for the detection of malignant pancreaticobiliary strictures<sup>[28]</sup>. Fluorescence in situ hybridization (FISH) has shown to increase the sensitivity for the diagnosis of malignant pancreaticobiliary strictures compared to conventional cytology. Kipp *et al*<sup>[29]</sup> used a multitarget FISH probe set which has previously shown high impact in monitoring recurrent urothelial carcinoma<sup>[30]</sup>. This advanced technique identifies malignant cells by detecting aneusomy and deletion of the locus 9p21. By applying this technique for brush cytology and bile aspirate specimens in 131 patients with bile duct strictures (including 71 with primary sclerosing cholangitis, FISH analysis showed sensitivity of 35% and specificity of 91%. When patients with primary sclerosing cholangitis were excluded, sensitivity for malignancy detection by FISH was 16%<sup>[31]</sup>. This indicates that probe sets specific for biliary neoplasms will be required for higher sensitivity. However, not all malignant tumors present aneusomy or aneuploidy. In the biliary tract, the percentage of cancers displaying aneuploidy has been estimated to be approximately 80%<sup>[32]</sup>. Since our current data clearly demonstrate that no improved sensitivity can be obtained by using forceps biopsy and/or brush cytology during endoscopic procedures, we therefore suggest that additional diagnostic techniques such as FISH techniques should be routinely evaluated in patients with negative cytological and histological findings.

## COMMENTS

### Background

Surgery is the only curative treatment in patients with hilar cholangiocarcinoma. The results are more favourable for patients with early-stage disease. Therefore, a reliable diagnostic procedure is of great importance in these patients. Cholangiocarcinomas often grow longitudinally along the bile duct rather than in radial direction away from the bile duct. Consequently, imaging techniques including ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI) are of limited sensitivity for the detection of cholangiocarcinoma. In patients with suspected malignant biliary stricture brush cytology and/or forceps biopsy were routinely used for distinction between benign and malignant strictures. Biliary tissue collection provides the only definitive diagnosis that can be used for establishing therapeutic strategies.

### Research frontiers

To our knowledge, no study exists investigating the sensitivity of brush cytology and forceps biopsy in a homogeneous group of patients with hilar cholangiocarcinoma. The current study was designed to address the question of diagnostic sensitivity for endoscopic transpapillary brush cytology and forceps biopsy in 58 patients with hilar cholangiocarcinoma.

### Innovations and breakthroughs

Positive results for malignancy were obtained in 41.4% of patients using brush cytology and in 53.4% of patients using forceps biopsy. The combination of both techniques brush cytology and forceps biopsy resulted only in a minor increase in diagnostic sensitivity to 60.3%. Consequently, in 23 out of 58 patients definitive diagnosis could not be achieved using the combination of brush cytology and forceps biopsy.

## Applications

Brush cytology and forceps biopsy have only limited sensitivity for the definite diagnosis of hilar cholangiocarcinoma. In our eyes, additional diagnostic techniques such as FISH techniques should be further evaluated and should become routine in patients with negative cytological and histological findings.

## Terminology

Cholangiocarcinomas are categorized as intrahepatic and extrahepatic carcinomas. Extrahepatic cholangiocarcinomas are further subdivided into hilar, medial and distal cholangiocarcinomas. The most common type is hilar cholangiocarcinoma which is also called Klatskin tumor. Klatskin tumors are classified into 4 stages according to the Bismuth classification: stage I or II for tumors expanding up the hilus, type III infiltrating right (III A) or left (III B) hepatic duct, and stage IV with infiltration of both hepatic ducts and sub segments.

## Peer review

The authors retrospectively analyze the effectiveness of various diagnostic tools to diagnose hilar cholangiocarcinoma. The authors come to the conclusions that brush cytology and forceps biopsy have only limited sensitivity for the definite diagnosis of hilar cholangiocarcinoma. The study suggest that in patients with suspected biliary stricture and negative cytological / histological findings further diagnostic approaches such as cholangioscopy should performed.

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