

Ethanol-based fixation is superior to conventional brush cytology in the evaluation of indeterminate biliary strictures by endoscopic retrograde cholangiography

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Abstract

The aim of this study was to compare the diagnostic yield of conventional cytology (CC) with ethanol-based fixation, a cytological analysis using an ethanol based fixative system including a cell block procedure (EBF) to evaluate indeterminate biliary strictures (IBStr). We also compared additionally taken fluorescence-guided forceps biopsies (FB) with EBF concerning a potential additive diagnostic benefit.

Early detection and accurate diagnosis are crucial for patients with suspected carcinoma within the biliary tree to preserve curative treatment options but diagnostics and patient care in the evaluation of IBStr are still challenging. ERC-guided brush cytology is the gold standard of nonsurgical evaluation of IBStr. However, accuracy is generally low. New specimen processing's are needed to higher the diagnostic yield in the evaluation of IBStr.

We performed a retrospective evaluation in 404 patients referred for further diagnosis of IBStr. Gold standard was defined as surgically obtained histology or patient follow-up of at least 1 year to diagnose or exclude malignancy.

Three hundred thirty-four patients were included into the final analysis. One hundred seventy-two strictures were malignant, 162 strictures benign. One hundred seventeen specimens were evaluated by CC, 217 processed by EBF. EBF performed significantly better in terms of sensitivity (24.6% vs 60%, $P < .001$) and accuracy (59.0% vs 75.1%, $P = .006$). Fifty-eight FB were additionally taken and showed a numerically improved sensitivity compared to EBF alone (80% vs 62.9%, $P = .19$).

EBF is a simple and inexpensive technique that substantially improved sensitivity and accuracy in the evaluation of IBStr. FB specimen did not significantly improve diagnostic yield.

Abbreviations: CBD = common bile duct, CC = conventional cytology, EBF = ethanol-based fixation, ERC = Endoscopic retrograde cholangiography, FB = forceps biopsies, FISH = Fluorescence in situ hybridization, IBStr = indeterminate biliary strictures, IDA = intraductal bile aspiration, IPMN = intrapapillary mucinous neoplasia.

Keywords: biliary tract neoplasms, brush cytology, endoscopic retrograde cholangiography, obstructive jaundice

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1. Introduction

Intra- and extrahepatic indeterminate biliary strictures with possible underlying pancreatobiliary malignancies remain a diagnostic challenge, despite of advances in improving endoscopic techniques and imaging. Most patients with pancreatobiliary malignancies are diagnosed in an advanced stage along with a poor survival rate.^[1] Therefore, early detection and accurate diagnosis are crucial for patients with suspected carcinoma development within the biliary tree to preserve curative treatment options. Moreover, the diagnosis of benign and malignant strictures is challenging since etiologies including postoperative strictures, chronic pancreatitis, primary, and secondary sclerosing cholangitis may present with cytologic atypia mimicking possible malignancy.^[2] This diagnostic dilemma leads up to ~7% to 9% of patients undergoing surgery because of suspected malignancy but ultimately diagnosed with benign diseases,^[3,4] putting these patients at risk for perioperative

morbidity and mortality. Therefore, accurate preoperative diagnosis of indeterminate biliary strictures is of utmost importance.

Endoscopic retrograde cholangiography (ERC) with brush cytology and/or endobiliary forceps biopsy is a routinely performed procedure of choice, widely available and associated with a low rate of procedure related complications.^[5] Even though imaging modalities like ultrasound and computed tomography or magnetic resonance imaging can be helpful in visualizing the tumor,^[6,7] ERCP with histology or cytology acquisition is still the gold standard to confirm malignancies, as defined by tissue diagnosis. Conventional brush cytology alone though has limiting low sensitivities ranging from 6% to 64% and overall sensitivity of $41.6\% \pm 3.2\%$, as described by Burnett and coworkers including 1556 patients.^[8] Using a combination of different techniques such as brush cytology, forceps biopsy, fine needle aspiration cytology or even more recent techniques like Fluorescence in situ hybridization (FISH) can improve the diagnostic yield.^[9–11] If, for example, triple tissue sampling is performed using biopsy, fine needle aspiration and brush cytology, sensitivity and specificity of 62% and 85% can be achieved, respectively.^[12] However, some of these approaches (e.g., FISH) are not widely available. Cytological smears are commonly used methods for pathological diagnosis. The diagnostic efficacy can also be significantly improved by modifying the pathological processing method.^[13]

The aim of this study was to compare the diagnostic yield of an ethanol-based fixative system including a cell block procedure^[14] compared to standard conventional brush cytology in a large cohort of patients with indeterminate biliary strictures. Although being widely available, EBF performance was not yet evaluated. Furthermore, double tissue sampling with additionally taken fluorescence-guided biopsies (from April 2011) was compared to EBF alone concerning a possible improvement in the diagnostic yield.

2. Materials and methods

2.1. Patients

Between February 2008 and March 2015, a total of 404 patients presented to the Interdisciplinary Endoscopy at Jena University Hospital for ERC with indeterminate biliary strictures for further evaluation. Except for acceptability of ERC and an age of at least 18 years, there were no exclusion criteria.

2.2. Endoscopic intervention and techniques

The endoscopic procedures were performed by faculties and attendants of Jena University Hospital, Clinic of Internal Medicine IV, who were highly experienced in pancreaticobiliary procedures. Tissue acquisition was performed with brush cytology and if feasible an additional fluorescence guided forceps biopsy was taken. ERC was performed using the standard technique with a single type of duodenoscope (TJF 180, Olympus, Tokyo, Japan). First, cannulation of the common bile duct (CBD) and endoscopic sphincterotomy were performed. After gaining access to the biliary tract, the “Cytomax II Double Lumen Cytologie Brush” (Wilson-Cook Medical Inc., Winston-Salem, Irland) was placed over a Jagwire .035/450 Guidewire (Boston Scientific Corporation, 300 Boston Scientific Way Marlborough, MA) inside its sheath under fluoroscopic guidance above the stricture. Once the brush was released out of its sheath, tissue sampling began moving it back and forth repeating this

maneuver with a minimum of five passages. Then the brush was pulled back into the sheath and pulled out as a single unit. Crossing the brush over the stricture was performed without prior dilatation of the stricture. If possible, a fluorescence guided forceps biopsy (Biopsy forceps, Jiangsu Kangjin Medical Instrument Co., Ltd; Zhenglu Town, Chang Zhou, China) was taken.

2.3. Final diagnosis and cytological examination

Gold standard was defined as a surgically obtained histology ($n = 140$) or a patient follow-up of at least 1 year to diagnose or exclude malignancy ($n = 194$). Patients with biliary strictures were considered benign if follow up of minimum of 12 month did not reveal any signs of malignancy.

Evaluation of cytological sample was performed by experienced pathology faculty from the Institute of Pathology, University Clinic Jena, Germany, without knowledge of the patient's history, laboratory results or prior imaging procedures. From February 2008, direct smear of specimen was performed on glass object plates by the endoscopist and directly send to the pathologist for further processing and diagnostics. From April 2011 methods were converted to EBF of cytology specimen including a cell block procedure to allow a histology-like processing of the cytological specimen.^[14] Ethanol-based fixation (BD CytoRich Red Preservative [Becton, Dickinson and Company, NJ]) preserves cells and small tissue fragments in suspension, lyses red blood cells and allows to perform immunohistochemical staining.^[14] Therefore, the brush was cut from its wire and directly placed into 5 mL ethanol-based fixative and send to the pathologist for further diagnostics and processing.

Cytological grading in five different categories was as follows: 0, non-diagnostic, insufficient; I, benign; II, atypical, favor benign; III, atypical, suspect malignant; IV, high grade dysplasia; V, tumor cells.

Results of cytology specimen were then divided into two groups, group A: category 0+I+II and group B: category III+IV +V. Group A was considered benign and B was regarded as positive for malignancy.

Forceps biopsies (FB) were also divided into two groups:

1. benign, if normal or signs of inflammation with possible underlying cell atypia were seen and
2. malignant, if atypia unaccountable through inflammation and therefore suspected malignant or carcinoma cells were present.

2.4. Statistical analysis

Results were described as median and range or mean and standard deviation. Statistical differences were calculated using Fisher's exact test and P -values $\leq .05$ were considered to be statistically significant. Results were calculated using the IBM SPSSwin Statistics software, version 24 (Somers, NY).

2.5. Ethical statement

The retrospective study was approved by the ethics committee of Jena University Hospital (No. 2019–1304-Daten). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the institution's human research committee. In accordance with

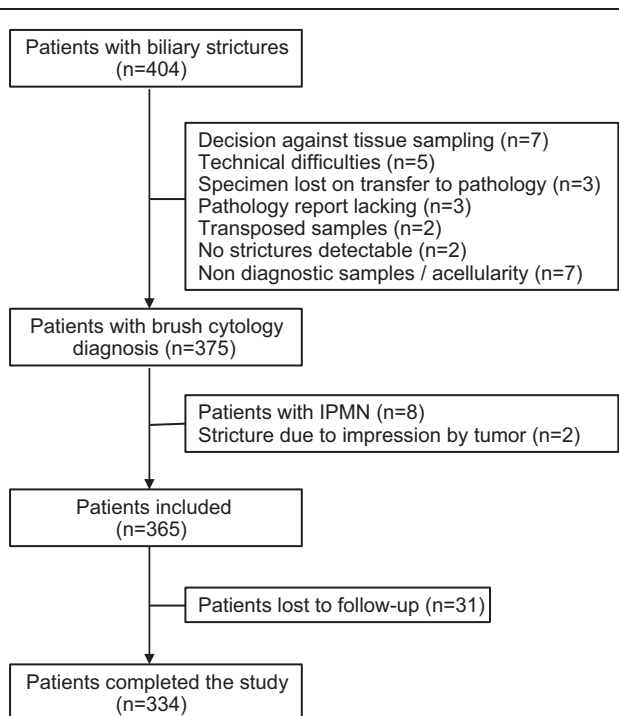


Figure 1. Flowsheet of patients.

German law, a written informed consent from the participants due to the strictly retrospective and anonymized design of our study (paragraph 27, sentence 2, Thuringian Hospital Act [ThürKHG] in the version of the notice of 15.06.2018) was not required.

3. Results

3.1. Patient characteristics

Four hundred and four patients presented for further diagnostic work-up. A total of 375 brushes were obtained: Two patients did not show any strictures. Three brushes got lost on the way to the pathologist and two specimens taken the same day got transposed and were excluded. Missing or lacking histology reports excluded another three specimens. Technical feasibility problems prevented five samples, samples were completely acellular in seven patients and in another seven cases the endoscopist decided against tissue sampling.

Of these 375 patients, eight patients with the final diagnosis of intrapapillary mucinous neoplasia (IPMN) were excluded due to not being the diagnostic tool of choice to diagnose these possible precancerous lesions. Furthermore, two patients with malignant strictures caused by impressions through chloroma and extramedullary tumor infiltration (multiple myeloma) with consecutive biliary strictures, were excluded in the final analysis of cytology performances.

Thirty-one of the remaining 365 patients got lost to follow up, therefore 334 patients could be finally evaluated (see Fig. 1). Patients were between 35 and 106 years of age (mean age was 68.7 years [SD 13.5 years], men represented 63% of the study population).

3.2. Characteristics of biliary strictures and final diagnoses

Localization of strictures were: intrahepatic (n=28), hilar (n=74), proximal CBD (n=35), distal CBD (n=173), papillary (n=24). One hundred seventy-two strictures were malignant, while 162 strictures were benign. Final diagnoses are summarized in Table 1.

3.3. Cytological results

Classification of specimen was as follows: category 0: 7 patients; category I–II: 236 patients, category III: 45 patients, category IV: 42 patients, category V: 11 patients. One hundred seventeen specimens were evaluated by CC, whereas 217 specimens were processed by EBF.

3.4. Comparison between EBF and CC

EBF in comparison to CC led to a significant improvement in sensitivity (60% vs 24.6% [$P < .0001$]) and accuracy (75.1% vs 59.0% [$P = .0028$]). Positive and negative predictive value also showed a tendency towards improvement even though not being statistically significant, while specificity remained considerably high (92.2%) (see Table 2).

3.5. Diagnostic yield of additional FB in addition to cytological specimen acquisition

In 58 cases fluorescence guided FB were additionally taken after EBF has been introduced in April 2011. These biopsies were taken upon the endoscopists assessment where technically feasible. Twenty-four FB were considered benign and 34 malignant. There was no significant difference in diagnostic

Table 1

Final diagnoses of all biliary strictures divided into malignant and benign diseases.

Malignant diseases (n = 172)		Benigne diseases (n = 162)	
Diagnosis	Number	Diagnosis	Number
Cholangiocarcinoma	81 (47.1%)	Pancreatitis	44 (27.2%)
Pancreatic cancer	46 (26.7%)	Miscellaneous	33 (20.4%)
Undifferentiated carcinoma	17 (10%)	Inflammatory	25 (15.4%)
Ampullary cancer	15 (8.7%)	Papillary stenosis	20 (12.4%)
Gallbladder cancer	11 (6.4%)	PSC/SSC	15 (9.3%)
HCC	2 (1.2%)	Anastomotic strictures	14 (8.6%)
		Caroli's disease	10 (6.2%)
		Lymph nodes	1 (0.6%)

HCC=hepatocellular carcinoma, PSC=primary sclerosing cholangitis, SSC=secondary sclerosing cholangitis.

Table 2
Test parameters comparing ethanol-based fixation (EBF) and conventional cytology (CC).

	All patients (%)	CC (%)	EBF (%)	P
Sensitivity	48.3	24.6	60.0	<.0001
Specificity	92.0	91.7	92.2	1
PPV	86.5	73.7	89.6	.1257
NPV	62.6	56.1	67.1	.1024
Accuracy	69.5	59.0	75.1	.0028

yield of EBF compared to biopsies within the same patient (see Table 3). Even though there was no significant enhancement of diagnostic yield using biopsies in addition to EBF, the combination showed a numerical improvement of sensitivity (80.0% vs 62.9%; $P = .1852$). Test parameters are summarized in Table 3.

4. Discussion

Accurate classification of benign versus malignant etiology of biliary strictures is of crucial importance with regard to further patient management. Brush cytology is routinely used at ERC to further elucidate the nature of indeterminate biliary strictures due to its easy use and broad availability. However, by use of standard cytological assessment sensitivity is rather low along with only modest diagnostic accuracy. In a Review by Burnett et al including 1,556 patients, CC alone showed an overall sensitivity of $41.6\% \pm 3.2\%$ (ranging from 6% to 64%).^[18] Other authors described similar broad ranges of sensitivities from 8% to 83%^[15,16] with consecutive high specificity, ranging from 90% to 100%.^[17] To preserve curative treatment options, to initiate neoadjuvant or even palliative therapy in case of malignancy and to prevent unnecessary surgery with potentially high morbidity and even mortality, it is of major importance to achieve better results from endoscopic tissue sampling.

In our study, we retrospectively investigated a large consecutive patient cohort with indeterminate biliary strictures according to the processing of cytological specimen, comparing an ethanol-based fixative system (“BD CytoRich Red Preservative”) to conventional brush cytology. To the best of our knowledge, this is the first study evaluating EBF in patients with IBStr. The most important finding of our study was that CC showed a low sensitivity of only 24.6%, but by using EBF sensitivity increased to 60% ($P < .0001$). In addition, specificity was preserved at 92.2% ($P = 1.0$), while PPV and NPV improved gradually, even if not statistically significant. Furthermore, EBF improved accuracy significantly from 59.0% to 75.1% ($P = .0028$).

There are several potential explanations for the substantially better test results using EBF: First, the use of the entire brush

Table 3
Forceps biopsies (FB) compared to ethanol-based fixation (EBF) and double tissue sampling compared to EBF alone.

	EBF (%)	FB (%)	P	EBF (%)	EBF plus FB (%)	P
Sensitivity	62.9	65.7	1	62.9	80.0	.1852
Specificity	91.3	95.7	1	91.3	91.3	1
PPV	91.7	95.8	1	91.7	93.3	1
NPV	61.8	64.7	1	61.8	75.0	.291
Accuracy	74.1	77.6	.8285	74.1	84.5	.2515

including all cells sticking onto it improved cellular yield as compared to a conventional smear cytology. Second, having a larger cell volume and through EBF protocol being able to assess the entire volume of obtained specimen, decreases a possible sampling error. Third, through lysis of additional and potentially disturbing red blood cells, it may have enhanced visualization of cells originating from the biliary endothelium/tumor and therefore facilitating a correct diagnosis. Fourth, until April 2011 various endoscopists prepared conventional slides by themselves, while using EBF specimen preparation was performed by (potentially more experienced) pathological faculty. Furthermore, EBF allows a pathological assessment similar to histological samples and the possibility for unlimited storage and molecular testing.

Final assignment of category III specimens to either a benign or a malignant category is still a matter of debate and histopathological interpretations especially in cases with suspicious results vary from one study to another. Therefore, close attention is needed when comparing the diagnostic accuracy of different reports.^[18] Although a more sophisticated categorization is scientifically well-founded,^[17] from a clinical perspective, assignment to the benign or malignant category is necessary for further decision-making. In a retrospective analysis by Weiler et al,^[19] a probability of malignancy in an “atypical category” for pancreatic and bile duct brushings of ~44% has been shown. Similarly, Eiholm and co-workers found malignancies in 80% of specimen categorized as atypical.^[20] Therefore, we a priori considered category III specimen as positive for malignancy similar to Howell et al,^[21] Jailwala et al^[12] and Kitajima et al.^[22] Regardless of which interpretation of category III will be decided, for example, counting category III to group A or evaluating this category alone, sensitivity and accuracy were always significantly improved by using EBF. Even when category III is not considered and only category IV and V in both cohorts are compared, EBF improves significantly sensitivity and accuracy (data not shown). Finally, ex post our clinical approach to interpret category III specimen as malignant has been confirmed by our study results, as 76.6% of category III specimen were malignant as defined by the gold standard.

Additional techniques of sample collection (e.g., forceps biopsy, intraductal bile aspiration [IDA], basket cytology) or a combination of cytological assessment tools may improve the diagnostic yield.^[5–7] Most authors recommend a combination of brush cytology with an additional technique, but which technology to be used is still a matter of debate: Ki Bae Bang et al recommend brush and basket cytology,^[23] Fior-Gozlan et al favor biliary brushings and bile aspiration,^[24] while Roesch T.,^[25] Ponchon T.,^[26] and Elek G. et al^[27] considered bile duct biopsies the gold standard. Unfortunately, advanced techniques such as FISH are not broadly available to be implemented in a daily clinical care. If brush cytology is combined with intraductal bile aspiration (IDA) remarkably high sensitivities up to 89% and 81% have been described.^[24,28] In our study additionally taken biopsies tend to yield higher sensitivity (80% vs 62.9%) and accuracy (84.5% vs 74.1%) despite being statistically significant. This lack of significance might be due to the low sample size ($n = 58$). Our findings support the results of a review by Navaneethan et al concluding that both brushings and biopsies are comparable and have limited sensitivity for the diagnosis of malignant biliary strictures and a combination of both only modestly increases sensitivity.^[29]

Our study has several method-inherent strengths and limitations. The retrospective nature of the study may have had an

impact on the results, as 17% of the consecutive patient population were not included in the final analysis. Another limitation is that endoscopists prepared conventional cytological slides, while pathologists performed EBF preparations. However, the latter aspect may also be considered as a method inherent strength of the EBF specimen processing.

One of the major strengths of our study is the large consecutive cohort of 334 patients representing a realistic patient population of daily clinical practice with a similar distribution of benign and malignant diseases. Compared to studies evaluating brushings and intraductal biopsies reviewed by Navaneethan et al,^[29] our study observed the largest patient population by far. Furthermore, during the study period all endoscopists remained the same which ensures a high constancy of the endoscopic procedures performed.

5. Conclusion

Our study suggests that EBF is a simple, universally available and inexpensive technique that yields better sensitivity and accuracy while preserving specificity compared to CC in patients with indeterminate biliary strictures. However, FB in addition to EBF improved diagnostic yield in our patient subgroup numerical, but not statistically significant.

Author contributions

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Analysis of cytologic specimen: Iver Petersen

Analysis of data: Martin Bürger, Carsten Schmidt

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