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ARTICLE

Early Diagnosis of Pancreatobiliary Duct Malignancies by Brush Cytology and Biopsy

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Two hundred and five preoperative intraductal samplings (brushing and biopsy) were evaluated from 113 patients with biliary or Wirsung duct strictures. One hundred and three strictures could be specified by histology of the operative specimens, autopsy, or by the patients' clinical course. Preoperative diagnostic efficacy depended on the tumor location (it was the best for ampullary and parapapillary tumors), but the average quantitative indices for sensitivity, absolute sensitivity, specificity, positive and negative predictive values, diagnostic accuracy of cytology were 53%, 20%, 100%, 100%, 25%, 59%, respectively. The same values for biopsy were 43%, 34%, 100%, 100%, 36% and 56%. These figures improved after simultaneous cytology and biopsy. Close cooperation with the endoscopist was necessary in cases of negative, inconclusive and dysplastic (27%) samples. Repetition of sampling improved the results by 8%. Among the 26 preoperative false negative cases, sampling-, technical- and interpretative errors occurred in 84%, 4% and 12%, respectively. Revision of samples revealed 4 malignant cases among the false negative cytologic brushings. Reclassification of specimens considering the latest criteria – primary and secondary malignant features, pancreatic intraepithelial neoplasia (PanINs), etc. – resulted in improvement of the diagnostic efficiency. (Pathology Oncology Research Vol 11, No 3, 145–155)

Key words: intraductal biopsy, intraductal cytology, pancreatobiliary strictures, sensitivity and specificity, sampling-, processing- and interpretive errors

Introduction

Endoscopic intraductal biopsy and brush cytology have become routine and accepted methods for diagnosis of strictures encountered during endoscopic retrograde choledocho-pancreatography (ERCP). The disadvantage of these methods is their modest sensitivity due to the relatively high occurrence of false negative results in malignant cases. The numerical indices of various endoscopic groups are variable.[13,18,21,47] The aim of this work is to present the preoperative histologic diagnostic efficacy of our group with these methods in all cases with stratified true diagnosis, i.e. the presence or absence of pancreatobiliary malignancy as established on the basis of autopsy and/or histology of the surgical specimen in a two-year period from 1999 to 2000.

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Materials and methods

During the two years 205 intraductal interventions were performed in 113 patients at the MÁV Hospital, Budapest. There were 56 men (mean age 67.0 ± 12.1 years, range 35-88 year) and 57 women (mean age 69.8 ± 11.1 years, range 45-92 year). The intraductal interventions without morphological samples are not included (*Table 1*). At ERCP after endoscopic papillotomy 113 intraductal cytology and 92 intraductal biopsy specimens were taken using radiologic guiding. In cases of simultaneous cytologic and biopsy sampling the forceps biopsy was attempted always after the cytologic brushing. The cytologic specimens were immediately transferred to ordinary glass slides by smearing the cellular material from the brush directly to the slides. The cytologic specimens were prefixed immediately with Cytology Fixative spray (Summamed, Gödöllő, Hungary), and after transfer to the Pathology Department (next day) fixed with equal mixture of ether-alcohol. The small tissue obtained by forceps biopsy was immedi-

Table 1. **Distribution of patients with intraductal samplings**

Technique of intraductal sampling	*Number of*			*Number of patients*						
				operated			*monitored only*		*total a+b+c +d+e*	*sub-tracted d+e*
	Pt	*S*	*V*	*only*	*+ M*	*A*	*less than two years*	*more*		
				a	*b*	*c*	*d*	*e*	*f*	*g*
Cytology	30	38		19	2	3	3	1	28	24
Biopsy	18	20	3	4	–	1	1	4	10	5
Cytology +biopsy	65	147	23	29	6	10	9	11	65	45
Total	113	205	26	52	8	14	13	16	103	74

A= autopsy, M= monitoring, Pt= patient, S= sample, V= Vater's papilla biopsy

ately placed into 10% buffered formalin solution and processed conventionally. Both the cytologic and biopsy samples were stained with HE and PAS. For biopsy samples, immunostaining with monoclonal DAKO anti-p53 serum was made in some cases, according to the indirect method using 3-amino-9-ethylcarbazole as chromogen on adhesive pretreated slides. More than 10% staining of epithelial nuclei was considered as positive.[12]

For biopsy specimens the histological diagnoses were conventional: 1. normal tissue or inflammation, 2. low- or medium-grade, 3. High-grade dysplasia, 4. carcinoma.[2,34]

A specimen was defined as adequate for cytologic diagnosis when on the two slides sufficient number of cells from the target site was obtained. For cytology the following categories were used: 1. negative for malignancy, 2. inconclusive (reactive or atypical, but not unequivocally benign), 3. suggestive for malignancy, 4. positive for malignancy. For purposes of data analysis, inconclusive, low- and medium-grade dysplasia diagnoses were considered as benign, and results that were suggestive for malignancy, and/or high-grade dysplasia were included in the positive or malignant category. For calculation of absolute sensitivity, only carcinoma and positive for malignancy categories were considered as malignant.[18]

The definitive diagnoses were established either by histopathology or through clinical follow-up. Histopathology and localization of tumors were obtained at autopsy or by surgical resection. When no histological findings were available, the following clinical criteria for malignancy were considered: radiological evidence of metastatic disease, rapid debilitation and death within 12 months, continued weight loss of >5 kg/month, persistent jaundice, or administration of chemo- or radiotherapy. Absence of carcinoma (benign stenosis) was considered in cases with maintenance of the patient's general status for two years after the intervention without jaundice and stent therapy.[23,31]

According to the definitive histology, the cytologic and biopsy results were retrospectively classified as true negative, true positive, false negative and false positive. The followings were calculated: sensitivity (percentage of specimens considered positive in patients with proven malignant disease), absolute sensitivity (percentage of specimens considered carcinoma or positive for malignancy in proven malignant cases), specificity (percentage of specimens considered negative in patients free of malignant disease), positive predictive value (percentage of positive results detecting the patients with malignancy), negative predictive value (percentage of negative results detecting the patients without malignancy), overall accuracy (percentage of correct positive and negative results in all patients under investigation)[13]. Because of the nature of this study involving retrospective analysis of false negative diagnoses, all cases were rescreened by a cytopathologist (GE) who was not blinded to the final diagnosis.[24] Dysplasia for cytologies and PanINs of some specimens were considered in the retrospective analysis only after reviewing the criteria described.[16,22]

Results

The clinical course of patients is shown in *Table 1*. Among 113 sampled patients, 103 could be evaluated (column *f*); 10 were lost due to the fact that they were referred from other institutions for the sampling procedure alone. Surgical interventions were carried out at several external surgical departments (52 cases, column *a*), therefore, complete follow-up was possible only for a minority of operated patients (8 cases, column *b*). Some patients were not operated on either because of their poor general status or due to the lack of certainty of the malignant nature of the stenosis (*c, d, e*). Some of them underwent autopsy in our hospital (14 cases, column *c*) or clinical monitoring until death. The latter patients had chemo- or

radiotherapy in other institutions and were observed there (13 cases, column *d*), therefore they were taken in consideration only in *Table 5*. Maintenance of general status of some patients led to the confirmation of the benign nature of the stenosis; from them control cases had been chosen (16 cases, column *e*).[19] We were unable to distinguish carcinomas of the biliary tract from those of the pancreas on the basis of cytomorphologic features alone, therefore, definitive localization of stenosis was not possible for group *d* and *e*. These had to be subtracted from the sum of patients, and thus 74 topographically localized tumorous cases remained (column *g*) without the nontumorous, true negative controls.

Table 2. **Sensitivity of methods with intraductal samples**

Tumor localization		*Vater's papilla*	*Pancreas*	*Para-papillar*	*Common bile duc*	*Klatskin tumor*	*All*
No. of patients		7	32	9	20	6	74
Cytology	AS	20%	14%	28%	20%	40%	20%
	IS	40%	38%	57%	30%	60%	39%
	SR		51%		35%		47%
	RS		59%		45%		53%
Biopsy	AS	40%	25%	44%	36%	33%	34%
	IS	20%	25%	55%	14%	33%	28%
	SR	40%	38%	66%	36%		43%
	P53	3/5	3/11	5/9	1/1		14/31
Both	AS	60%	30%	40%	35%		36%
	SR	60%	54%		50%	50%	49%
	RS		69%	71%	57%		64%
False negative*		1/7	11/32	1/9	8/20	1/6	22/74
OS		85%	66%	89%	60%	83%	70%

AS = absolute sensitivity. IS = initial sensitivity: malignant from the first intervention. SR = sensitivity including repeated interventions with the same intraductal method. RS = revised sensitivity: all + reclassified cytologies after two years learning phase. OS = overall sensitivity: malignant by any kind of intraductal intervention. *Malignant only intraoperatively or after autopsy.

Sensitivity can be seen in *Table 2*. Some patients had only cytology or biopsy samples, while others had both. The number of patients with both cytology and biopsy is less than in *Table 1* (39 as opposed to 45), since in six cases these interventions were not simultaneous. The sensitivity of intraductal biopsy was lower than that of cytology in this material, it was only 43% as compared with the 53% of cytologic results. Absolute sensitivity values (carcinoma diagnoses without severe dysplasia or suggestion of malignancy) were generally lower for cytology than for biopsy. Repeated interventions resulted in better sensitivity for both cytology and biopsy (by 8% and 15%, respectively). Revision of cytological specimens revealed four false negative cases which could have been ranked malignant. This resulted in a 6% increase in sensitivity of cytology. By combining intraductal cytology and biopsy, sensitivity could be increased by an additional 6-21%. Tumors of various locations differed from each other in diagnostic sensitivity. Percentages for pancreas and common bile duct tumors were the lowest values, but they were comparable with each other. False negative values remaining after the combined methods are shown in the last rows. The overall sensitivity was calculated on the basis of these false negative results. The overall 70% sensitivity allowed early diagnosis in 18 cases out of 74 (24%) in whom no imaging method (either abdominal CT or ultrasonography) pointed to malignancy, and only the intraductal cytology or histology confirmed the suggestion of ERCP. Positive p53 staining of biopsies appeared in 50% of cases. Among them only one case was positive when the diagnosis with HE and PAS seemed to be inflammation. Cytological samples were not immunostained for p53, because the smears were not made on adhesive slides and their number was limited.

As no false positive case was found, the specificity and the positive predictive values were 100%. The percentages of negative predictive values and the diagnostic accuracy are presented in *Table 3*. The data show that the negative predictivity of intraductal cytology is somewhat lower than that of the biopsy, but its accuracy exceeds that of biopsy. Combined application of intraductal biopsy and cytology resulted in more advantageous negative predictivity and accuracy than the isolated use of any of them.

Various types of errors can be best analyzed in the group of patients having both intraductal cytology and biopsy. For this purpose not only the simultaneously tested 39, but all the consecutively tested patients (11 controls and 45 tumorous cases, see *Tables 1, 2* and *3*) were considered. Diagnostic results of brushing and biopsy are not always the same. The 56 patients were divided in three groups. In

Table 3. **Negative predictive values** (NPV) **and diagnostic accuracy** (DA) **of methods with intraductal samples.**

Tumor	*Vater's papilla*	*Pancreas*	*Para-papillar*	*Common bile duct*	*Klatskin tumor*	*All localizations*	*True negative, controls*	
Cytology	25%	21%	25%	27%	33%	25%	10	NPV
	50%	63%	63%	54%	67%	59%		DA
Biopsy	40%	33%	25%	40%	33%	36%	15	NPV
	57%	52%	70%	55%	50%	56%		DA
Both	50%	43%	33%	45%	50%	44%	11	NPV
	75%	75%	75%	68%	67%	72%		DA
Overall*	50%	21%	50%	38%	57%	33%	11	NPV
	88%	67%	90%	68%	86%	74%		DA

Positive predictive values and specificity were 100%. Nontumorous (control) cases were chosen from *Table 1*, column e. *Overall: including all cases by any method.

the first group (20 patients) the cytology and biopsy suggested similar diagnosis. In the second group (14 patients) the biopsy, while in the third one (22 patients) the cytology showed more definitively a malignant alteration (*Table 4*). False negative results are in the second row. In our hand the brushing was more sensitive for detecting malignancy than the intraductal biopsy, but biopsy was safer for excluding it; the negative predictive value of the biopsy was higher than that of cytology. In the last column all investigated cases are presented (16 controls and 74 tumorous cases, see *Tables 1* and *2*). As revised cases are classified here as errors, the number of false negative results are not 22, but 26.

From the revised cases the interpretive and technical errors can be estimated. The 4 reclassified cases (all cytologic samples) were the following brushings: one mucinous intraductal pancreatic carcinoma (*Figure 2c*) and two cases suggestive of malignancy (*Figures 3d* and *4c*). These brushings were diagnosed originally as inconclusive. After reclassification they became equivalent to high-grade dysplasia (one of them in the pancreas, PanIN 3). The fourth reclassified carcinoma was overlooked because of imperfect (too strong) nuclear staining of the brushing (*Figure 1d*), it can be regarded as technical error. The percentage of the interpretive error was 5.4% of all cases. The percentage of inconclusive diagnoses among all the 95 cases having brush cytology was 27% (*Table 5*).

One additional case is a technical error, too. In this case the biopsy was lost during processing, but the simultaneous cytology was successful, and thus false negativity has been avoided. Technical error occurred in 1.8% of all cases.

The cellularity of cytologic samples was variable, ranging from scanty to hypercellular. Four of ten hypocellular cytologic slides belonged to the false negative cases, corresponding to 7.1% overall. Also, one third of the sampling errors could be due to hypocellular brushings.

In the second group two additional patients had acellular smears, and the diagnoses were positive only due to the tumor-containing biopsies. Thus the minimal number of sclerotic neoplasms is at least two (3.6%) and the maximum possible number of subepithelially spreading or sclerotic malignancies *(Figure 1b*) may be 14, all from the patients in the second group (25%). The frequency of errors is often expressed as percentage of false negative cases. Considering the foregoing calculations, the sampling error was about 84%, the interpretive error around 12%, and the technical (processing) error 4% or more of the 26 false negative cases in our material. Based on our experience we suggested a practice of the microscopic diagnosis on brushings, summarized in *Table 6*.

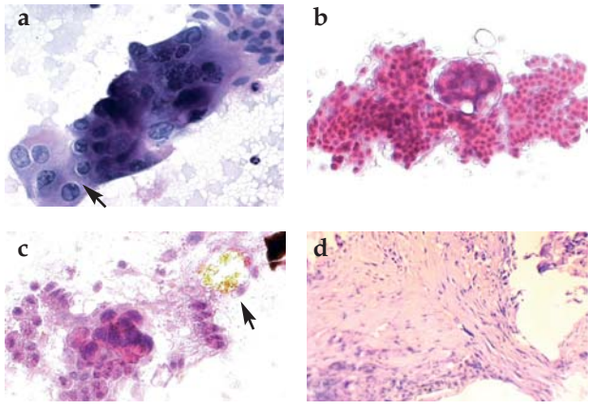

Figure 1. *Normal* and tumorous ductal epithelium side by side in the brushings.* ***(a)*** *Chromatin of the tumorous nuclei is irregularly distributed, coarse (chromatin clumping, arrow). PAS, 650x.* ***(b)*** *Small cluster of tumor cells with large nuclei (arrow) appears in one luminal border almost surrounded with normal cells. HE, 200x.* ***(c)*** *Reactive atypia of normal cells due to bile concretion (arrow). HE, 650x.* ***(d)*** *Anisonucleosis, loss of polarity and striking hyperchromasia point to malignancy but granulocytes to reactive atypia. HE, 650x.*

Table 4. **Diagnostic errors in cancer patients tested with intraductal methodology and/or cytology and biopsy**

	Diagnosis			*Total*	*All patients (tested with one method)*
	Same with both methods	*More serious by*			
		Biopsy	*Cytology*		
Case number	20	14	22	56	90
False negative	6	3	6	15	26
Brushing only (hypocellular)	2	1	1	4	10
revised	0	0	3	3	4*

*Interpretive and technical error together

Discussion

Since the 1950s, cytology and endoscopic biopsy have become an established method for obtaining tissue diagnosis in many clinical areas (cervix, breast, lung, gastrointestinal and urinary tract, etc.).[5] In the case of biliary and pancreatic tract strictures, only exfoliated cells from bile and pancreatic juice were used for such purposes, with variable success. Endoscopic retrograde brush cytology was first reported in 1970,[29] and endobiliary forceps biopsy in 1978.[21] These methods became widely adopted at about the turn of the 20th and 21st century. The delay was due mainly to the technical difficulties of obtaining adequate samples. Therefore, the first reports have emphasized the technical aspects with diagnostic usefulness of these procedures.[13,21,22,47] Brush cytology proved significantly better than exfoliative bile or pancreatic juice cytology, because the samples of the former are more cellular and less autolysed.[9,21,25,31,40,49] The sensitivity was greater in patients with cholangiocarcinoma than in those with pancreatic cancer,[13,21,24,40] similarly to our preliminary results.[14] This difference was explained by the difficulties in reaching the diagnostic area of lesions of the pancreas.[26] With good equipment and experience, however, the accuracy of pancreatic brush cytology may be similar to that of biliary cytology.[44]

Sensitivity depends on the technique, selection of patients, the site of origin of the malignancy, type of neoplasm, the experience of endoscopist and pathologist, and the quality of cytologic material.[28,32] Treating severe dysplasia as equivalent to malignancy will spuriously increase sensitivity („best case") and decrease specificity. On the other hand, dysplasia or atypia included in the benign category will underestimate sensitivity („worst case"), but increase specificity.[17,18,23,44] For this reason, absolute and complete sensitivity should be distinguished: the former excludes dysplasia, while the latter includes it. The best absolute sensitivity values are around 40 % in the literature.[18] For cytology our 20% absolute value is low, because at the learning curve we were very protective for high specificity at the expense of modest sensitivity, in agreement with the opinion that the pathologist should be conservative in rendering malignant diagnosis on this particular type of cytologic specimens.[9,18] Complete sensitivity was reported between 30-70% for years,[18,21,22] similarly to our 50% value (*Table 2*). The upper limits of this interval have hardly grown in the last ten years, except for a few cases of surprisingly high, 80% sensitivity, mentioned for biliary tract brushing.[13,47] The sensitivity and specificity values in various articles are usually the complete ones. Diagnostic sensitivity of both cytology and biopsy is the best for ampullary and parapapillary tumors, similarly to our material.[30,35]

There appear to be several reasons for the relatively low diagnostic sensitivity of ERCP-directed brushing. Outside the domain of cytopathologist, endoscopic sampling error seems to be the major cause of false negative diagnoses (60 - 80% of them).[6,24] There are two sorts of this error. (a) In certain cases the brush will not bring out sufficient material for analysis and the smears are hypocellular or acellular. There is no way to check for that possibility of failure, except if an immediate interpretation can be performed by a cytologist.[7,30] The presence of a cytopathologist at the intraductal sampling, however, can not be always guaranteed. A possibility to avoid this pitfall is maximizing cellular yield at cell collection. In some

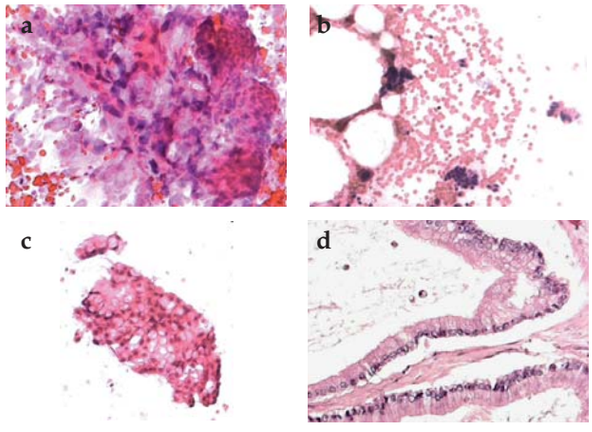

Figure 2. *Brush cytology of mucinous carcinomas.* ***(a)*** *Moderately differentiated mucinous carcinoma. HE, 200x.* ***(b)*** *Smear from poorly differentiated infiltrative mucinous carcinoma with small discohesive clusters with macronuclei (asterisk). HE, 200x.* ***(c)*** *Intraductal papillary mucinous neoplasm. HE, 200x.* ***(d)*** *Biopsy of differentiated, infiltrative papillary mucinous carcinoma. HE, 200x.*

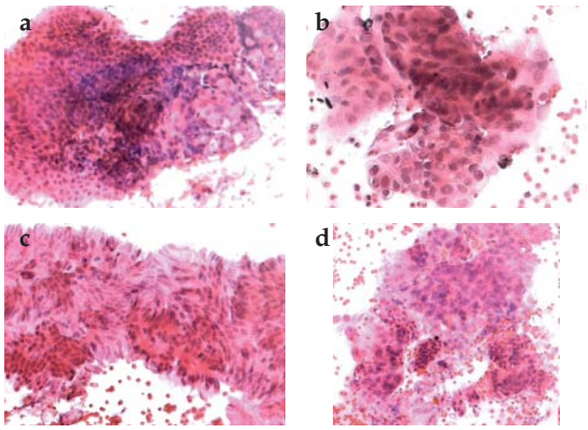

Figure 3. *Spatially aggregated epithelium: reactive, dysplastic and malignant.* ***(a)*** *Reactive change. HE, 200x.* ***(b)*** *Ductal cells of chronic inflammation. HE, 400x, inset 200x.* ***(c)*** *Papillomatosis. HE, 200x.* ***(d)*** *Pancreatic cancer. HE, 200x.*

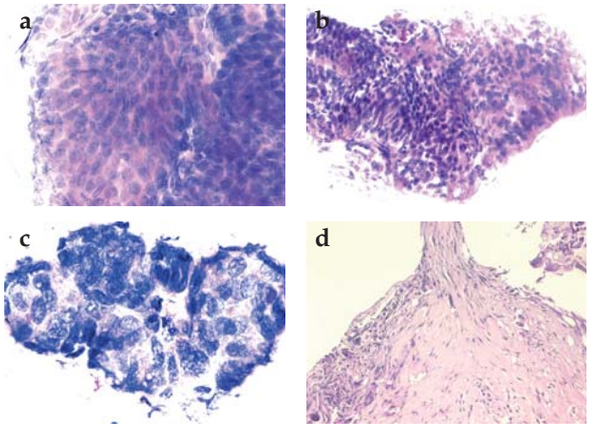

Figure 4. *Folded shape of the cell cluster, multicentric orientation of nuclei may point to papillarization: reactive, dysplastic and malignant.* ***(a)*** *Reactive (artificial) papillarization. PAS, 650x.* ***(b)*** *Cytology of medium-grade dysplasia. HE, 200x.* ***(c)*** *"Unnoticed" single cluster of cells in PanIN3. PAS, 650x.* ***(d)*** *Intraductal biopsy. Deep ductal tumorous infiltration (at the low left corner). HE, 100x.*

places, after smearing on slides the rest of the cells is washed from the brush and cytocentrifuged.[22,24] Smears may be hypocellular because of inaccessible anatomic sites (ampulla, genu and the tail of Wirsung).[26] In cases of tight stenosis it is difficult to take samples directly from the tumor sites (desmoplastic cancer, reactive fibrosis, sclerosing cholangitis or pancreatitis).[50] This problem might be encountered in malignant stenoses of other parts of the digestive system. Sampling procedure requires a high degree of skills by the endoscopist. Perhaps brushes of various types with bristle stiffness are needed.[17] (b) In other cases brushing does not contain malignant cells. The origin of the stenosis might be extraductal (metastatic or secondary carcinoma, gallbladder or hepatocellular cancer, lymph node metastasis or lymphoma, islet cell or acinar cell tumor of the pancreas).[29] Sometimes a benign epithelium overlying the malignant stricture is the cause of sampling error (*Figure 1b*). Sampling may be difficult because of poor visualization. Intraductal specimens are obtained at fluoroscopic guidance, which is two dimensional. Lack of direct sight makes separation the florid and necrotic part of tumor impossible, and the brushing may contain overwhelming necrotic cells. In such instances intraductal endoscopic biopsy or ultrasound-guided endoscopic fine needle aspiration are the most sensitive diagnostic methods.[6,7] Although sampling errors are generally recorded outside the domain of the pathologist, for better patient care good communication with the endoscopist should be provided.[18] A negative diagnosis should be viewed with more skepticism, and the possibility of repeated examination should be considered. Repeated brushings are likely to identify malignancy if the ERCP suggests a malignant stricture, thus contributing to improved sensitivity and diagnostic accuracy. In our case, a second intervention raised the sensitivity by 8% in average (*Table 2*). After 3 consecutive negative endoscopic brush cytologies the chance of a malignant biliary stricture may be less than 6%.[23,24,30-32,41] In any suspicion of sampling error, communication with the endoscopist is essential for further management of the patient.[3]

Technical error is around 10-20% of false negative cases. This includes all suboptimal preparations resulted by inappropriate cytotechnical processing. Almost all authors underline the importance of air-drying artifact, which interferes with assessment of virtually all cytologic features, precluding an accurate interpretation, therefore, fixation is critical.[24,25,33] Technical error in our material was around 4% without air drying artifact due to the immediate prefixation with fixative spray after brushing. We call attention to another sort of technical error, suboptimal nuclear staining, which makes the evaluation of malignant chromatin difficult (*Figure 1d*). Some authors prefer washing the cells from the brush (and subsequent centrifugation: liquid phase preparation) to direct smearing,[28] but according to others, direct smearing from the brush is as good as washing.[25] Some architectural features are different in brushing and liquid phase preparations (honeycomb).[47]

Interpretive error occur in case of unrecognized malignancy in the specimen. Few studies have discussed the cytologic evaluation of pancreatobiliary brushings in detail.[9,18,47] Although the morphology of pancreatobiliary fine needle aspiration is different in some respect (less necrosis, more fibrous tissue, fragments of vessels, etc.), it can be easily applied to direct brushings.[3,11,45] Diagnostic criteria for cytomorphological assessment of material from

Table 5. **Incidence of dysplasia in pancreatobiliary brushings (%)**

Reference	*Patients (no.)*	*Dysplastic cases (no.)*	*Dysplastic cases (%)*
Ponchon et al[30]	204	24	12
Layfield et al[22]	108	20*	19
Lee et al[23]	149	32	21
Kocjan et al[18]	131	10	8
de Peralta et al[9]	104	25	24
Mansfield et al[25]	54	9	17
Glasbrenner et al[13]	115	9	8
Vandervoort et al[44]	143	38	27
Stewart et al[36]	143	19	13
Jailwala et al[17]	133	14	11
Stewart et al[35]	406	41	10
Henke et al[15]	419	158*	38
Ylagan et al[47]	142	9	6
Okonkwo et al[28]	139	36	26
Present series	95	26	27

*Including reactive, inflammatory atypia

the pancreaticobiliary tree are in principle not different from those in other areas. The complexity, however, can exceed that of other epithelial systems, because the criteria that are most useful in differentiating benign strictures from biliary tract carcinoma or from pancreatic carcinoma may be different[15] (*Figure 3d*). In spite of this, the origin of the sample is difficult or impossible to discern by routine cytologic examination or from small biopsy specimen.[6,9,21] Frequency of errors was estimated around 8-17% of the false negative cases,[24] which is comparable to ours (12%). Serious interpretive error arises when technical errors are neglected or not recognized, and the pathologist attempts to make a definitive diagnosis based on limited or poorly preserved cellular material[3]. Special types of carcinomas may appear banal. It is a hard task to separate a mucinous hyperplasia from well-differentiated mucinous carcinoma (*Figures 2, 3a*), or papillomatosis from papillary adenocarcinoma (*Figures 1a, 3c*), or, more importantly, from normal reactive epithelial clusters (*Figures 3a, 4a*).

Dysplasia is another important area of potential diagnostic pitfalls. No samples with dysplasia were reported in earlier publications.[21,26,32] Later the „atypical" (inconclusive) category was introduced, when the cytological criteria fell closer to a definitive diagnosis of malignancy.[3] This few percentage of cases were either omitted from, or included in the calculation of sensitivity,[32 31,44] depending on the benign or malignant outcome of the majority of the patients. The numbers of atypical diagnosis were 10-30% of all investigated cases in the last years (*Table 5* and *Figures 3c, 4c,b*); ours was 27% for brushed cases. The term dysplasia was proposed for brushings in 1995,[22] and have been used by some authors[13,18,23,24] but not by others.[15,28,35,36] The reasons why the term atypia was used instead of dysplasia are the followings. (a) The definitions of dysplasia suggested[22] were subjective and overlapped with criteria for benign, reactive and malignant changes, therefore, were difficult to reproduce.[28] (b) Brush cytology is limited in distinguishing dysplasia and adenocarcinoma[6,25,35] especially for ampullary tumors. (c) Histological criteria for dysplasia of pancreatobiliary malignancies are not definitively settled.[15] There are new histological definitions,[2,34] recently the PanINs.[16] These latter need further refinement for application to brushings (*Figures 3c, 4b,c*).

Since severe dysplasia carries an increased risk for cancer, it is important to report its suspicion in brush cytology.[24] To be more practical, however, these diagnoses should be minimized, because they present a management problem for the gastroenterologist.[28] Better interpretation includes awareness of some benign pathological conditions specific to this area (*Figure 1c*). Sclerosing cholangitis, chronic pancreatitis, or stones are well recognized to either coexist with or predispose to malignancy, but none of them have specific cytologic findings. Even in surgical specimens the distinction between sclerosing cholangitis and cholangiocarcinoma can be extremely difficult for the pathologist, and the patient's course can sometimes be more informative.[30,50] Diagnostic pitfalls may be avoided if the cytological diagnosis of dysplasia is regarded by both the clinician and the cytopathologist as a useful diagnostic category reflecting the natural history of neoplastic processes in this area, rather than a diagnostic uncertainty.[15,18] Dysplasia is strongly suggestive of malignancy, the probability of which is appreciable for the individual patient.[23] This represents another group of patients for whom close cooperation between pathologist and endoscopist is indispensable.

Cytologists should develop a skill for assessing the overall degree of atypia or abnormality in a specimen. There are many signs of malignancy (about twenty),[8] and even if when only a few of them are present, the malignant pattern can be recognized (*Figure 1*). This process is called „overall assessment of malignancy", a reproducible determination of the presence of carcinoma. This explains that sensitivity of cytology may increase from the initial to the final period of investigations by 5-30%.[18,35] In our case the „revision" resulted in about 6% improvement. It was thought advisable to identify the key cytologic features (primary or major criteria), the most useful ones in distinguishing benign strictures from adenocarcinoma of the pancreatobiliary tract.[8,33] As the frequency of some signs is different in various types of carcinoma, the primary criteria for biliary and pancreatic malignancies are different.[15] The presence of two or three of the major criteria in the examined specimen guarantees 98% specificity and 83%

***Table 6. M*icroscopic diagnosis of pancreatobiliary brushings (summary)**

				Grade of the dysplasia			
		Normal	*Reactive*	*low or medium*	*high*	*Cancer*	*Source of diagnostic errors*
group	Cellular	homogenous	not homogenous	might be homogenous	small number of atypical groups +	large isolated cells	source of the sample is not known
shape	Cellular	polarized	polarized	cuboid form recognizable	cuboidal form lost, cell membrane hardly discernible		sampling errors, hypocellular brushings
arrangement	Cellular	oriented	disturbed	multipolarly arranged	overlapped, crowded, folded sheet, cribriform		sampling errors, hypocellular brushings
shape	Nuclear	oval	slightly pointed	slightly elongated	irregular, may be molded		suboptimal fixation (drying artifact) and nuclear stain
membrane	Nuclear	undiscernible	hardly discernible	discernible	irregular, cytoplasmic inclusions		suboptimal fixation (drying artifact) and nuclear stain
chromatin	Nuclear		fine	irregular	may be roughly clumped		suboptimal fixation (drying artifact) and nuclear stain
size	Nuclear	similar	fine variance	slight variance	wide variance, usually large		submucosal growth of the tumor and no biopsy
arrangement	Nuclear	on the same level		pseudostra-tified	multicentrically arranged irregularly stratified		submucosal growth of the tumor and no biopsy
nucleolus		not visible	may be seen	discernible	sometimes multiple		submucosal growth of the tumor and no biopsy
subjective sources of the diagnostic errors		missing the scale relations of the normal cells when examining patho-logical material	uncertainty in the differentiation of the spatial lesions reactive atypia	not recognizing rare malignant cells among inflam-matory or necrotic elements	papillary formations	missing the diagnosis of intraductal mucinous tumors	imperfect contact with the clinician, and endoscopist

sensitivity. Reclassification of dysplastic cases by supplementary major criteria may result in a further 5-20% improvement of sensitivity.[28,47] Also, the critical review and such a reclassification can explain the previously mentioned higher (~80%) sensitivity in recent series.[9,15]

The intraductal forceps biopsy remained in the second line of investigation.[6,21] The range of sensitivity is 30-60%, depending on the sequence of its application in relation to brushing. Our 43% sensitivity is modest, but higher values were reached only in series when biopsy preceded cytology,[32] multiple or larger samples were taken by malleable forceps in selected patients,[20,30] or the process was guided by intraductal ultrasonography.[10] Almost all authors agreed that combination of cytology with biopsy enhances diagnostic yield by 10 to 20%.[17,20,30] The increase in our case was 15% in average (*Table 2*). Biopsy and cytology have a complementary role in the definitive diagnosis of dysplasia and in correcting sampling error (*Figures 3c, 4*). In cases of cytologic sampling of a desmoplastic tumor, ampullary villous adenoma, and at suspicion of extraductal stenosis, the simultaneous biopsy may be particularly useful for excluding or revealing an underlying invasive process.[6,12] Differentiated mucinous tumors may demonstrate marked architectural atypia on biopsy specimen as contrasted with small and minimally atypical nuclei precluding a definitive diagnosis of malignancy on cytological samples[9] (*Figure 2c,d*). If the biopsy is too small, superficial, distorted, fragmented, inflamed or necrotic, these architectural criteria of malignancy might be difficult to recognize. The smaller rate of the intraductal biopsy due to technical difficulty makes brushing specimen often the only pathological material available. This is why despite its variable sensitivity, brush cytology remains the major diagnostic modality in patients with pancreaticobiliary strictures.

The high degree of false negativity have led to search for ancillary techniques that could improve the diagnostic accuracy. Detection of K-ras codon 12 mutations in pancreatic carcinoma may be a valuable adjunct to the classic light microscopy of brush cytology.[39] It requires, however, specialized equipment and expertise for PCR-based identification, not suitable on a large scale within a clinical setting. p53 immunocytology raised the sensitivity in some investigations,[42] but not in others.[31,36,43] The 50% positivity of malignant biopsies in our present series was less than that found in Vater's papilla biopsies.[12] The rarely observed false positivity can be explained by microscopic dysplastic foci in Wirsung duct, also common in chronic inflammation.[4] For this reason p53 positivity suggests a malignant tumor only in cases of simultaneously demonstrable high-grade dysplasia.[12] Although the molecular methods are not generally used for routine diagnosis, they may be indispensable in difficult cases.[37]

The results of fine needle aspiration cytology for biliary and pancreatic lesions were claimed to be inferior to those for other tumors.[17,21] In the case of bile duct carcinoma the method may be limited because of the focal and sclerotic nature of this particular neoplasm. The fine needle aspiration is only as good as the ability of imaging to detect a focal pancreatic lesion, which in this location may not be early enough for considering radical operation.[46] Ultrasound- or computer tomography-guided percutaneous aspiration, or even transhepatic cholangioscopy[41] have the risk of intraperitoneal spread of tumor, or other complications,[7,20,21] and the sensitivity is inferior than with the endoscopic brushing.[9] Such interventions are not indicated in radiographically resectable pancreatic tumor.[27] Endoscopic ultrasound-guided or intraluminal fine needle aspiration have high sensitivity and the probability of peritoneal tumor spreading is minimal, however, these methods need special equipment, considerable time and expertise.[7,17] The utility of all these methods is the avoidance of a more invasive intervention, leading to substantial cost saving. If the diagnostic efforts are unsuccessful but the patient has a high clinical suspicion of malignancy with a potentially resectable lesion, an exploratory laparotomy should be considered. In this situation fine needle aspiration cytology performed at laparotomy is useful for obtaining a tissue diagnosis before resection. Intraoperative sampling may be more sensitive than some preoperative techniques,[11,21,22,46] because of better visualization of dislodging tumor cells mainly with intraoperative ultrasonography.

In gynecologic exfoliative cytology, which is one of the most advanced systems (Bethesda), the precancerous cytologic terms are in accordance with histological terms, and are significantly reproducible and interchangeable. This is, however, the outcome of the practice for many decades. ERCP with cytology and biopsy of pancreaticobiliary malignancies will most likely make a similar development, but at present we are only at the beginning of this route.[48] Selection of major (primary) and minor (secondary) cytologic criteria of malignancy[15,28] or definition of histologic signs of pancreatic in situ neoplasia (PanINs[16]) are the first steps towards the reproducibility of early tissue diagnosis. Further studies would be required, however, to improve the cytologic criteria of dysplasia in various types of pancreaticobiliary malignancies. Significant reduction in the number of false negative diagnoses can be achieved only by development of strict diagnostic criteria, and by producing specimens that are technically perfect with no sampling error.

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