Brush cytology of ductal strictures during ERCP

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Abstract

Background : **Previous reports on endoscopic retrograde brush cytology (ERBC) of bile ducts and of the main pancreatic duct have reached widely varying sensitivity levels of 33 up to 85%.**

Aims : **To report our experience with ERBC in a series of biliary strictures (n = 98) and pancreatic duct strictures (n = 8). For the purpose of our study, that was mainly directed to the value of the cytologic examination as such, only those specimens that were considered satisfactory for cytological interpretation were studied.**

Patients : **From October 1988 until August 1994, 154 cytologic brushings were performed at ERCP in 132 patients. In 132 brushings obtained from 115 patients (86%), cell yield was satisfactory for cytologic interpretation. Nine patients lacked adequate follow-up. Hence, 123 brushings from 106 patients were included in this study. A final diagnosis of malignancy was obtained in 62 patients.**

Methods : **Cytological changes were described as 'benign', 'columnar cell intraepithelial neoplasia', 'inconclusive' by the presence of atypical cells, or 'malignant'.**

Results : **For a positive diagnosis of the malignant nature of a stenosis, ERBC had an overall sensitivity of 63% with a specificity of 96%. One false positive result was obtained in a patient with a biliary infection by Fasciola Hepatica. Sensitivity was highest in malignant ampullary strictures (91%). Sensitivity was 60% for cholangiocarcinomas, and 65% for pancreatic cancer. The finding of 'columnar cell intra-epithelial neoplasia' in the ampullary region led to a Whipple resection and diagnosis of invasive carcinoma in one patient. Atypical cells were found in 4 brush specimens : in three of these 4 cases, a malignant lesion proved to be present.**

Conclusions : **Brush cytology is a simple technique with a high specificity and should be performed in biliary and pancreatic duct strictures of unknown etiology. Categorizing the smears according to cytomorphology may improve diagnostic accuracy.** (Acta gastroenterol. belg., **2000**, 63, **254-259**).

Key words : biliary tract diseases, biliary tract neoplasms, cytology, dysplasia, cholangiocarcinoma, ampulla of vater.

Introduction

During endoscopic retrograde cholangio-pancreaticography (ERCP), strictures of the bile ducts and/or of the main pancreatic duct are often found. The nature of the obstruction cannot be diagnosed conclusively in all cases based on radiographic findings alone. To establish a definitive diagnosis, cytological or histological confirmation is needed. In abrasive cytology, such as obtained at endoscopic retrograde brush cytology (ERBC) of bile ducts and of the main pancreatic duct, highly cellular samples are frequently obtained, consisting of well-preserved cells (1). It is the purpose of the present study to report on our experience with the use of ERBC as a diagnostic tool in a large series of patients with strictures of the extra- and intrahepatic bile ducts (n = 98), or of the main pancreatic duct (n = 8).

Patients, materials and methods

From October 1988 until August 1994, 154 endoscopic brush cytology specimens were obtained from 132 non-consecutive patients with strictures of the extrahepatic or intrahepatic bile ducts or of the main pancreatic duct. Ninety patients presented with jaundice. Other presenting symptoms consisted of right upper quadrant pain (n = 39) and fever (n = 20).

ERBC was only performed when a correct diagnosis could not be made based on endoscopic and radiologic data alone. All endoscopic procedures were performed by a single endoscopist, using an Olympus TJF20 duodenoscope or a TJF100 videoduodenoscope. From 1988 until 1991, an Olympus cytology brush set (BC-19Q), and from May 1991 till August 1994, the Geenen cytology brush set (GCB-200-3-1,5, Cook) was used. The method described by Scudera *et al.* (2) was followed. The brush was then removed through the sheath and smeared onto glass slides. Salvage cytology was not performed, so that a significant loss of cellular material may have occurred (3). Cell samples were immediately fixed in 95% alcohol. The slides were stained according to Papanicolaou. For the purpose of this study, all samples were reviewed by a single cytopathologist, without any knowledge of clinical data or previous cytopathologic results.

One hundred and thirty two brushings from 115 patients were considered satisfactory for cytological interpretation (86%) : cell yield was sufficient and artefacts due to excessive pressure during smearing or to delayed fixation were minimal. Nine patients were excluded since no adequate follow-up was available. Hence, 106 patients, 62 males and 44 females with a mean age of 62 years (range 26-92 years), from wich 123 representative cytologic samples were obtained, were included. Ninety-two patients underwent one, 11 patients two, and 3 patients underwent three brush cytologies from the same lesion at repeated endoscopic examinations. Malignancy (invasive cancer) was proven in 62 patients (58%). This final diagnosis was established by histo- or cytopathologic (other than ERBC) diagnosis of the primary tumor or of metastases in 24 patients (39%), by a clinical course with death due

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to exhaustion or development of liver metastases in 23 patients (37%), by surgical exploration in 6 patients (10%), or by later ultrasonography and/of CT-scan in 9 cases (14%). Benign strictures were present in 42 patients (40%). The diagnosis was proven by the clinical course with a follow-up ranging from 4 to 6 years, by repeated investigation with ERCP, CT, and blood tests, or by surgical exploration. In two patients (2%), a histologic diagnosis of dysplasia was obtained.

In 25 patients, either a clearcut stenosis of the ampullary region or a suspicion of an ampullary problem because of biliary symptoms and unexplained dilatation of the bile duct system was present. Although, admittedly, the traditional way to diagnose an ampullary problem is the performance of biopsies after sphincterotomy, it was decided to use brush cytology also for this region in an attempt to reach the whole ampullary mucosa and not just the sphincterotomy margins.

Cytologic findings were considered 'within normal limits' when sheets and/or strips of normal-appearing columnar epithelial cells of the duct lining were present. 'Reactive changes' included nuclear enlargement, slight anisokaryosis, multinucleation, and the presence of a round, vesicular nucleus with a macro-nucleolus. Both categories, 'normal findings' and 'reactive changes' were grouped together as 'benign findings'. When atypical cells were present but no differential diagnosis could be made between reactive and neoplastic changes, cytologic findings were considered 'inconclusive'.

'Columnar cell intra-epithelial neoplasia' (CCIN) was diagnosed when strips of columnar cells were present, showing pseudostratification of the elongated, slightly enlarged, hyperchromatic nuclei. Our category of 'columnar cell intra-epithelial neoplasia' corresponds to the dysplastic changes found at pancreatic and biliary duct brushing, as described by Layfield *et al.* (4).

The diagnosis of 'carcinoma' was based on architectural and cytological alterations. Three- dimensional cell groups with nuclear crowding, and with tubular and/or papillary configurations were present. Epithelial cells were columnar or polygonal and contained an enlarged, hyperchromatic, elongated or rounded nucleus with indentations of the nuclear membrane, irregularly distributed chromatin and a prominent nucleolus. Cellular and nuclear pleomorphism were evident.

When most of these changes were present, brush cytology was considered 'clearly malignant'. When alterations were limited, brush cytology was considered 'very suspicious for malignancy'. For the purpose of this study, both categories 'clearly malignant' and 'very suspicious for malignancy' were grouped together as malignant changes.

Results

Of the 25 strictures localised in the ampullary region, from which a total of 30 brushings were obtained, 9 proved to be invasive adenocarcinoma, one corresponded to a carcinoid tumor of the ampulla, and 13 to a benign stenosis of the papilla. Two lesions were characterized by the presence of dysplasia (Table 1). Brush cytology revealed 'malignant changes' in 8 out of 11 brushings obtained in patients with invasive cancer. Two repeated brushings in a patient with a later surgically proven ampullary adenocarcinoma showed the picture of 'intra- epithelial neoplasia'. This patient presented with recurrent attacks of biliary colic and cholangitis, and showed a mild dilatation of the common bile duct at ERCP. No endoscopic or radiologic arguments for tumor were found. Based on the results of brush cytology, a pyloruspreserving Whipple resection was performed. Histologically, severe dysplastic changes and signs of invasive carcinoma were found in the region of papilla ; dysplastic changes were also found at the section margin of the pancreatic resection specimen. Overall, the sensitivity of brush cytology in malignant ampullary strictures was 91% (10 positives out of a total of 11 brushes). ERBC was signed out 'benign' in all benign lesions. 'Inconclusive' cytological findings were obtained in the patient with the ampullary carcinoid tumor. A final diagnosis of dysplasia was made in one patient by histological examination of ampullary biopsies, and in another patient in whom a tubular adenoma was found at Whipple resection. On the total of 3 brushings obtained in these 2 cases, one showed the picture of 'intra-epithelial neoplasia'.

Sixty-eight strictures were localized in the extrahepatic bile ducts. Twenty-one strictures were benign whereas 47 proved to be malignant (Table 1). Cytologic investigation revealed 'malignant changes' in 29 out of 51 brushings taken in patients with malignant lesions. ERBC was signed out 'benign' in 28, and 'inconclusive' in one out of 29 brushings obtained in patients with benign diseases. This results in an overall sensitivity of 57% and a specificity of 100%. In one patient who was found to have a cholangiocarcinoma, a second brushing revealed malignant cells whereas a first brush specimen had been signed out as 'benign'. Sensitivity for detection of cholangiocarcinoma and for pancreatic cancer was 61% (17/28 brushings) and 59% (10/17 brushings), respectively.

Of the 5 strictures localized in the intrahepatic bile ducts, 3 corresponded to benign and 2 to malignant lesions. Benign diagnoses included one case of infestation with Fasciola Hepatica, one polycystic disease of the liver, and one non-specific stenosis in a patient with lithiasis and cholangitis. Malignant lesions corresponded to intrahepatic cholangiocarcinomas (Table 1). ERBC of benign strictures was signed out as 'benign' in two cases. It was false positive in the patient with Fasciola Hepatica infestation, in whom atypical cells 'very suggestive for malignancy' were found. Cytologic investigation revealed 'malignant changes' in one out of two cases with cholangiocarcinoma.

Table 1. — Results of "well-readable" endoscopic retrograde brush cytology (ERBC) specimens in different regions of the biliary tree and in the pancreatic duct system

Final diagnosis	Patients	Brushings	Results of ERBC			
	N	N	Malignant N (%)	Benign N (%)	Inconclusive N (%)	Intra epithelial neoplasia N (%)
Ampullary						
Invasive carcinoma	9	11	8 (73)	1 (9)	0	2 (18)
Carcinoid tumor	1	1	0	0	1	0
Dysplasia	2	3	0	2	0	1
Benign stenosis	13	15	0	15	0	0
Total	25	30	8	18	1	3
Extrahepatic Bile ducts						
Cholangiocs	26	28	17(61)	11 (39)	0	
Pancreatic ca	16	17	10 (59)	7 (41)	0	
Metastasis	1	1	0	1	0	
Gallbladder ca	3	3	2	0	1	
Lymphoma	1	2	0	2	0	
All malignancies	47	51	29 (57)	21 (41)	1 (2)	
Benign stenosis	21	29	0	28	1	
Total	68	80	29	49	2	
Intrahepatic Bile ducts						
Cholangioca	2	2	1	0	1	
Benign stenosis	3	3	1	2	0	
Pancreatic duct						
Pancreatic ca	3	3	3	0	0	
Benign stenosis	5	5	0	5	0	
All cholangioca's	28	30	18 (60)	11 (37)	1 (3)	
All pancreutic ca's	19	20	13 (65)	7 (35)	0	
All malignancies	62	68	41 (60)	22 (32)	3 (4)	2 (3)
All benign lesions	42	52	1 (2)	50 (96)	1 (2)	

Eight strictures were localized in the main pancreatic duct, corresponding to 5 benign and 3 malignant lesions. Benign diagnoses included two pseudocysts, one cystadenoma, and chronic pancreatitis. Brush cytology was signed out 'benign' in all five benign strictures. It revealed 'malignant changes' in all three cases of invasive carcinoma (Table 1).

As summarized in Table 1, malignancy was diagnosed by ERBC in 10 out of 1 1 brushings obtained from primary malignant lesions of the ampulla (91%), in 18 out of 30 brushing specimens obtained from cholangiocarcinomas of the intra- or extrahepatic bile ducts (60%), and in 13 out of 20 specimens (65%) obtained in patients with pancreatic carcinoma. For all brushing specimens that were obtained from malignant lesions, overall sensitivity was 63% (43 positives out of 68) ; taking all specimens obtained from benign strictures into account, specificity was 96% (50 signed as as benign out of 52 specimens). The positive predictive value of a "malignant" cytology was 98%, the negative predictive value being 69%.

Discussion

Strictures of the biliary and panereatie ducts that are demonstrated at ERCP or at percutaneous transhepatic cholangiography, may be caused by a variety of benign and malignant diseases. The distinction between malignant and benign lesions is crucial to define prognosis as well as management strategies.

Therefore, much attention has been payed to various diagnostic endobiliary cytotechniques which can conveniently be performed during ERCP. Cytologic investigation of bile aspirated from the common bile duet, or of pancreatic juice collected after seeretin stimulation is charaeterized by a low sensitivity of 6-30% (1,5-8). Indeed, it is well known that the cell yield in exfoliative cytology is usually low and that spontaneously exfoliated cells are often poorly preserved, rendering cytologic interpretation difficult (1). Cytologic analysis of the cellular debris on stents removed from patients who were managed with these devices yields a sensitivity (36%) similar to that of brush cytology (33%), but is impractical because the diagnosis is delayed until the endoprosthesis is removed (8,9). Endobiliary biopsy methods have been developed, either with a malleable biopsy forceps (10,11), or with a pre-bent ball-tipped catheter with a retractable 22-gauge needle to obtain endoscopic needle aspiration biopsies (ENA) from biliary strictures (11,12). Using the malleable biopsy forceps, Kubota *et al.* (10) reported an overall sensitivity of 81% for the diagnosis of bile duct and pancreatic carcinoma

in a consecutive series of 43 patients with pancreaticobiliary ductal strictures. Howell *et al.* (12), using ENA in a consecutive series of 31 patients with biliary strictures, found an overall sensitivity of 62% for the diagnosis of malignancy, including 53% of patients with pancreatic cancer and 80% of those with cholangiocarcinoma. In the latter study, results of ENA were far superior to those of brush cytology. However, only 2 out of 24 brushings (8%) performed in patients with malignant stenoses were positive. Of all available endobiliary cytotechniques, experience is greatest with brush cytology. Previous reports on endoscopic retrograde brush cytology of bile ducts and of the main pancreatic duct have reached overall sensitivity levels of 33% to 85% (2,5,7-9,11,13-26) with, in most series, a specificity of 100% (Table 2).

The present series reports on 123 representative brush cytology specimens obtained from 106 patients during ERCP. Several comments have to be made on the way the results are presented. Twenty-two brush cytology specimens were considered unsatisfactory and have purposefully been omitted for calculating sensitivity and specificity. The analysis consisted of a "cytology oriented approach" and was mainly carried out to investigate the value of well-readable cytology specimens. Along the same way, sensitivity and specificity results are presented as "percentages of brushings" and not as "percentages of patients". In order to comply with the "intention to diagnose" principle, we should have included all patients with biliary and pancreatic duct strictures during the period under study, and we should have included all patients who underwent the procedure, not just those who had a satisfactory brushing specimens. It is clear that our "well-readable cytology approach" may overestimate the real sensitivity of brushing cytology as an endoscopic technique.

Taking these considerations into account, overall sensitivity of a "well-readable brushing specimen" for diagnosis of a malignant stenosis was 63%, with a specificity of 96%. One false positive report was obtained in a patient with a Fasciola Hepatica infection, in whom markedly atypical cells were found due to severe inflammation and atypical repair. Sensitivity may vary depending on the localization of the stricture and on the type of malignant lesion. As can be seen from Table 2, that summarizes the results of endoscopic brush cytology of 12 series, sensitivity is similar for detection of cholangiocarcinoma (62%) and of pancreatic cancer (59%), but is markedly higher in ampullary adenocarcinoma (78%). Similarly, in our study, sensitivity of brush cytology was 91%, 60%, and 65% for invasive carcinomas of the ampullary lesion, of the bile ducts, and of the pancreas, respectively. Sensitivity is lowest in metastatic invasion of the bile ducts. On a total of 43 patients with a metastatic bile duct stenosis, 17 had a positive brush cytology (40%) (2,11-13,15,18,20,23). Metastatic tumors that initially extrinsically compress the bile duct or manifest submucosal growth are unlikely to be detected by brush cytology during the early stage of the disease, whereas cholangiocarcinomas that originate from the ductal epithelium are more amenable to cytologic diagnosis (9).

In our series, atypical cells were found in 4 brush specimens, rendering cytological findings 'inconclusive'. In three out of these 4 cases, a malignant lesion proved to be present, consisting of a carcinoid tumor of the papillary region, a gallbladder carcinoma, and a cholangiocarcinoma, respectively. The fourth patient proved to have chronic pancreatitis with distal common bile duct stenosis, without any evidence of malignancy during a five-year follow-up. Including the presence of atypical cells as a positive result should have increased sensitivity from 63 to 68%. In the series of Wiersema *et al.* (11), the presence of atypia on cytology or biopsy specimens was 100% predictive of a malignant lesion. In Ryan's experience, markedly atypical cells, inconclusive for a diagnosis of malignancy, were identified in 36% (9/25 brushings) of patients with false negative cytology results, whereas these findings were not seen in patients with benign diseases (18). The small number of patients with inconclusive cytology in our series is undoubtedly related to the selection of the brushing specimens.

In our total series of 123 brushings, three cytologic specimens showed a picture of 'intra-epithelial neoplasia', which was characterized by the presence of columnar cells with pseudostratification of their elongated, slightly enlarged, hyperchromatic nuclei. Layfield *et al.* (4), in their paper on the morphological aspects of pancreatic and biliary duct brushings, reported on the finding of low and high-grade dysplastic changes, characterized by mild to prominent nuclear enlargement, nuclear crowding and overlapping, an increased nuclear/cytoplasmic ratio, coarse chromatine, and distinct to prominent nucleoli. In our opinion, the latter changes correspond to our finding of 'columnar cell intra-epithelial neoplasia'. In contrast, however, with our low prevalence of 'intra-epithelial neoplasia' in only three out of 106 patients, dysplasia was found in 23% of 168 consecutive bile duct brushings in 149 patients, described by Lee *et al.* (24). In one of our three patients, a definite diagnosis of invasive carcinoma was made at surgery. According to Lee's experience, 10 out of 13 patients with highgrade dysplasia and 10 out of 19 cases with low-grade dysplasia had a malignant lesion (24), suggesting that the cytological evidence of dysplasia in biliary or pancreatic duct brushing specimens should alert the clinician to the possibility that a significant lesion is present. In a recent series of Thonke *et al.* (26), all 3 patients with dysplasia found at brush cytology proved to have a malignant lesion. Categorizing dysplasia under malignant cytology increased their sensitivity for the detection of malignancy from 77 to 81%.

Table 2. — Cumulative results of endoscopic retrograde brush cytology (ERBC)

Authors	Patients and localization of strictures					Overall results				Sensitivity according to tumor type and localization			
	Total N	Malignant N (%)	Bile duct N	Wirsung N	Sens %	Spec %	PPV %	NPV %	Bile duct %	Wirsung %	Chol-Ca N (%)	Pan-Ca N (%)	Amp~Ca N (%)
Desa (5)	24	10 (42)	24	0	50	93	83	72	50	–	5/10 (50)	–	–
Ferrari (13)	74	52 (70)	55	19	56	100	100	51	54	64	2/10 (20)	19/29 (60)	1/1 (100)
Kurzawinsk (7)	46	3g (85)	46	0	69	100	100	37	69	–	6/10 (60)	15/23 (65)	5/5 (100)
Foutch (15)	34[a]	24 (62)	34	5	54.	100	100	58	55	50	6/6 (100)	3/5 (50)	–
Rabinowitz (7)	65	37 (57)	65	0	62	100	100	67	62	–	23/37 (62)	–	–
Ryan (18)	69[b]	45 (73)	52	13	44	100	100	44	49	30	4/9 (44)	6/20 (30)	4/4 (100)
Scudera (2)	25	20 (80)	25	0	60	100	100	38	60	–	2/2 (100)	5/10 (50)	5/7 (71)
Venu (20)	53[c]	42 (84)	n.r.	n.r.	69	100	100	42	n.r.	n.r.	20/25 (80)	3/5 (60)	–
Sawada (21)	72	72 (100)	0	72	85	n.r.	n.r.	n.r.	–	85	–	61/72 (85)	–
Ponchon (22)	193	127 (66)	127	0	35	97	96	44	35	–	12/25 (44)[d]	3/20 (15)[d]	5/9 (56)
Pugliese (23)	94	64 (68)	94	0	54	100	100	50	54	–	16/22 (73)	8/24 (33)	9/11 (82)
Wiersema (11)	29	25 (86)	n.r.	n.r.	55	100	100	29	n.r.	n.r.	7/11 (64)	4/9 (44)	–
Total	778	557 (72)	522	109	58	99	98	48	55	66	103/167 (62)	127/217 (59)	29/37 (78)

a : A total of 39 strictures in 34 patients ; sensitivity of 60% when calculated for the number of patients with cancer diagnosed by ERBC

b : Successful brushing obtained in only 62 patients (90%)

c : Successful brushing obtained in only 50 patients (94%)

d : Successful cytology and final diagnosis obtained in 193 out of total of 233 patients who underwent an attempt at brush cytology and/or endobiliary biopsy ; sensitivity according to tumor type only given for lesions with final diagnosis proven by surgery or autopsy

Sens : sensitivity ; spec : specificity ; PPV : positive predictive value ; NPV : negative predictive value ; chol-ca : cholangiocarcinoma ; pan-ca : pancreastic cancer ; amp-ca :ampullary carcinoma ; n.r. : not reported.

Endoscopic series on brush cytology to date have yielded wide variations in overall sensitivities. According to Lee *et al.* (24), this might partially be related to the methodology used, to the prevalence of malignancy in the study subjects, to the inappropriate analysis of cytological dysplasia, and to the prospective or retrospective character of the studies. Low sensitivities of 33-37% have been found in some prospective studies on consecutive patients (8,22,24), but not in other similar studies (7,20,23). To increase the sensitivity of brush cytology in biliopancreatic strictures, supplemental techniques have been proposed such as the combination of brush cytology with transpapillary fine needle aspiration (ENA) (11,12), or with endobiliary forceps biopsy (11). In the experience of Wiersema *et al.* (11), sensitivity of brush cytology in patients with malignant biliary strictures increased from 55% to 67% and 73% if combined with ENA or with forceps biopsy, respectively. A new device has been developed permitting placement of all three types of sampling devices over a guide wire without requiring sphincterotomy (27). According to Mohandas *et al.* (28), diagnostic yield with exfoliative bile cytology may markedly be increased by dilation to 10 French gauge with disruption of biliary strictures. If results of a first brushing are negative, a second and third attempt at a later time are indicated to obtain better results. In the series of Rabinovitz *et al.* (17), the probability of having bile duct carcinoma after three sequential negative cytologic brushings was below 6%, whereas sensitivity of the first brushing was only 40%.

It can be concluded that brush cytology is a reliable method for the diagnosis of malignancy in patients with biliary or pancreatic strictures documented at ERCP. A benign result of a single brush examination cannot be accepted with confidence. At the present time, it is not clear what combination of methods yields the best results Further prospective studies are needed to compare different diagnostic strategies.

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