Coll. Antropol. **34** (2010) 1: 139–143
Original scientific paper

Biliary Brush Cytology for the Diagnosis of Malignancy: A Single Center Experience

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A B S T R A C T

Differentiation between benign and malignant biliary strictures is critical to the provision of adequate treatment. Brush cytology during the endoscopic retrograde cholangiopancreatography (ERCP) is the most commonly used method for obtaining tissue confirmation of the nature of biliary strictures. It's specificity is remarkably high but reported sensitivities for the diagnosis of malignancy are low. Aim of our study was to assess sensitivity and specificity of biliary brush cytology in our institution, to find out main causes of false negative diagnoses and to confirm impression that the team approach has impact on sensitivity. Gold standard for diagnosis was definitive surgical histology or adequate clinical follow up for minimum of six month. Direct smears made by cytotechnician at the endoscopy room, and stained according to Papanicolaou and May-Grünwald Giemsa (MGG) were examined for well-recognized features of malignancy on conventional smears as a part of diagnostic routine. Cytologic diagnoses were benign, atypical/reactive, suspicious for malignancy and malignant. Of 143 brushings with available definitive diagnosis 36 (25%) had malignant cytologic diagnosis and 91(63.6%) were classified as benign, 3 were atypical/reactive and 13 suspicious for malignancy with 20 »false-negative« cases. When specimens with atypical and suspicious cytology were excluded from data analysis sensitivity was 64% and specificity was 100% and when suspicious findings were taken into account as true positives sensitivity rose to 71%. We find that biliary brush cytology, although mainly depending on the skill of endoscopist, as well as the experience of the cytologist, is a valuable method for obtaining accurate tissue diagnosis of biliary strictures, thus solving eternal diagnostic dilemma: benign or malignant.

Key words: *biliary malignancy, pancreatic malignancy, biliary brush cytology, endoscopic retrograde cholangiopancreatography (ERCP)*

Introduction

Malignant biliary strictures can be difficult to distinguish from benign conditions during endoscopy and despite new diagnostic tools preoperative diagnosis of biliary strictures remains a challenge[1–3]. Accurate differentiation between benign and malignant processes is critical to the provision of adequate treatment, so the importance of tissue based diagnosis of biliary strictures is well recognized[4]. The differential diagnosis includes but is not limited to: primary sclerosing cholangitis, gallbladder carcinoma, pancreatic carcinoma, intraductal papillary mucinous tumor or benign postimmflamatory strictures. Majority of pancreatobiliary malignant neoplasms are carcinomas but other malignant tumors can be encountered.

Brush cytology during the endoscopic retrograde cholangiopancreatography (ERCP) is most commonly used method for obtaining tissue confirmation of the nature of biliary strictures. Since its introduction[5] many studies have shown that brush cytology during ERCP is useful diagnostic method which is simple to perform, does not increase rate of complications and has potential of obtaining definitive diagnosis, aiding in further patient management. Its specificity is remarkably high but the

Received for publication July 31, 2009

main complaint about the method is its low sensitivity for the diagnosis of malignancy. Most of the studies report sensitivity 30–54% for bile duct brushings and 26–88% for overall brushings of pancreatobiliary tract[6]. Low sensitivity is commonly attributed to the high rate of false negative diagnosis. Sampling errors and technical reasons such as air-drying artifacts have been reported as main reasons for high rate of false negatives[7]. Better communication and team approach are said to have impact on sensitivity also[8]. Kocjan et al. found four main categories of reasons responsible for low sensitivity of biliary brushings including sampling error, dysplasia, special tumor types and smear background[9].

Aim of our study was to assess sensitivity and specificity of biliary brush cytology in our institution. Reviewing false negative cases we wanted to identify main causes of false negative diagnoses and tried to recognize which of mentioned factors influenced cytologic diagnosis the most. We also wanted to confirm impression that the team approach has impact on sensitivity as reported.

Material and Methods

During the five years period (between November 2003 and December 2008) 201 brushings from 178 patients with lesion of the pancreato-biliary system, including papilla, were taken during the ERCP procedure and submitted to our cytology department. For the purpose of this study only patients with definitive diagnosis were considered. Inadequate samples, 3 of them, were also excluded from analysis. Gold standard for diagnosis was definitive surgical histology or adequate clinical follow up for minimum of six month. Follow up was not possible for some patients referred from other institutions for ERCP procedure alone, but for 143 samples complete data were available.

Sample collection and smear preparation

Depending on the amount of collected material, for each patient 2–5 direct smears were made by cytotechnician present at the endoscopy room. At least one smear was immediately fixed in 95% ethanol for Papanicolaou staining and the rest were air-dried and subsequently stained according to May-Grünwald Giemsa (MGG). In rare cases immunocytochemistry was performed.

Specimens were examined for well-recognized features of malignancy on conventional smears as a part of diagnostic routine. Considering well-established criteria, cytologic diagnoses were categorized as benign, atypical/reactive, suspicious for malignancy and malignant[9–15].

Statistical analysis was performed using MedCalc for Windows, version 10 (MedCalc Software, Mariakerke, Belgium).

Results

From a total of 201 biliary brushings gold standard diagnosis was obtained for 143 specimens from 119 patients. There were 34 females and 85 males, mean age was 62 years (range 20–89).

Of 143 brushings with available definitive diagnosis 36 (25%) had malignant cytologic diagnosis and 91 (63.6%) were classified as benign. However, 20 out of 91 patients with initially benign cytologic diagnosis had subsequent malignant diagnosis established either by FNA, surgical specimen or clinical course, so they were considered as false negatives (Table 1). Main malignant diagnosis was carcinoma (Figure 1) and there was one case of lymphoma (Figure 2) and one metastatic melanoma found in brushing specimens. Twelve patients had repeated procedures in the course of several months and ten of them remained negative for malignancy. Two cases diagnosed as malignant in repeated cytology, had initially less than satisfactory specimens and were repeated immediately after negative diagnosis. There were 3 cases (2%) diagnosed as atypical/reactive and 13 (9%) specimens were suspicious for malignancy.

When cases with cytologic diagnosis of atypia and suspicious for malignancy were excluded from data analysis (leaving 127 specimens), absolute sensitivity was 64% and specificity was 100%. Of thirteen suspicious diagnoses, 12 had hystological diagnosis of carcinoma on subsequent surgery and one case was diagnosed as dysplasia gravis on biopsy specimen. For this study this case was considered false positive. All atypical cases were shown to be benign on follow up (Table 1). When thirteen suspi-

TABLE 1
CYTOLOGIC AND DEFINITIVE DIAGNOSIS IN 143 BILIARY BRUSH SPECIMENS

	Definitive diagnosis		
Cytology	Benign	Malignant	Total
Benign	71	20	91
Atypical	3	0	3
Suspicious	1	12	13
Malignant	0	36	36
Total	75	68	143

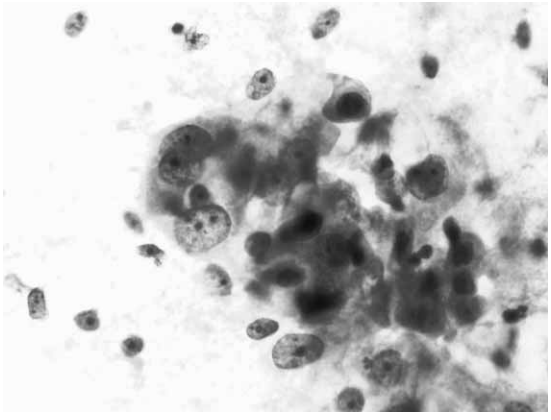

Fig. 1. Group of clearly malignant cells,sufficient for diagnosis of carcinoma (Papanicolaou stain, x1000).

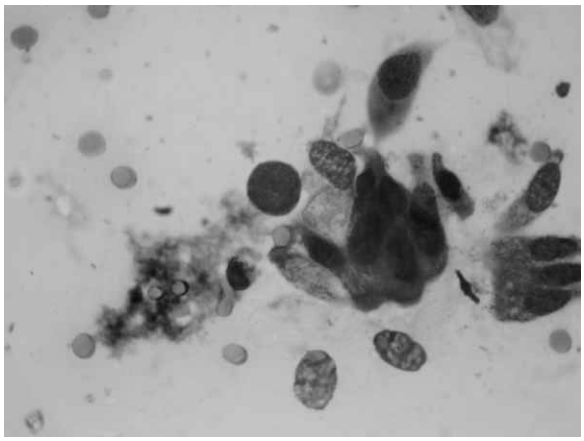

Fig. 2. Non-Hodgkin lymphoma in biliary brushing specimen (MGG stain, x1000).

cious cases were combined with cases positive for malignancy into a single category, as is customary, sensitivity rose to 71% and specificity dropped to 98.7%. Carefully reviewing 20 false negative cases, we found malignant cells present on the slides in only one case.

Discussion and Conclusion

Most of the studies report sensitivity of only 30–54% for bile duct brushings and 26–88% for overall brushings of pancreatobiliary tract[6,11,16]. Although those results are not directly comparable due to the fact that some of them refer to specific pathology (high sensitivity being reported for detection of cholangiocarcinoma in the special setting of primary slerosing cholangitis[10]) and different use of statistical tests[6,17,18], their common denominator is relatively low sensitivity[7,19–22]. Reasons for low sensitivity have been discussed[6], and according to Kocjan et al. they fall into several categories including sampling error, dysplasia, special tumor types and smear background. We don't regularly use the term dysplasia in our laboratory, and the term suspicious is reserved for specimens that meet some criteria for malignancy but we were not confident to make definitive diagnosis, usually due to poor preservation of cells or low number of undoubtedly malignant cells on the slide.

Absolute sensitivity of biliary brush cytology (excluding suspicious and atypical findings from data analysis) in our institution was 64%, somewhat higher than in most reported studies. With suspicious findings included in malignant category, sensitivity rose to 71% with only a minimal drop of specificity. Urbano and his group showed that the team work and close cooperation of cytopathologist and gastroenterologist have an impact on sensitivity of the method. In our institution cytotechnician is always present in the endoscopy room at the time of the procedure, smears are made directly on the glass slides and relevant clinical informations are obtained. We believe that this kind of preparation of cytologic material has positively influenced sensitivity of the method and is probably the reason for low proportion of inadequate

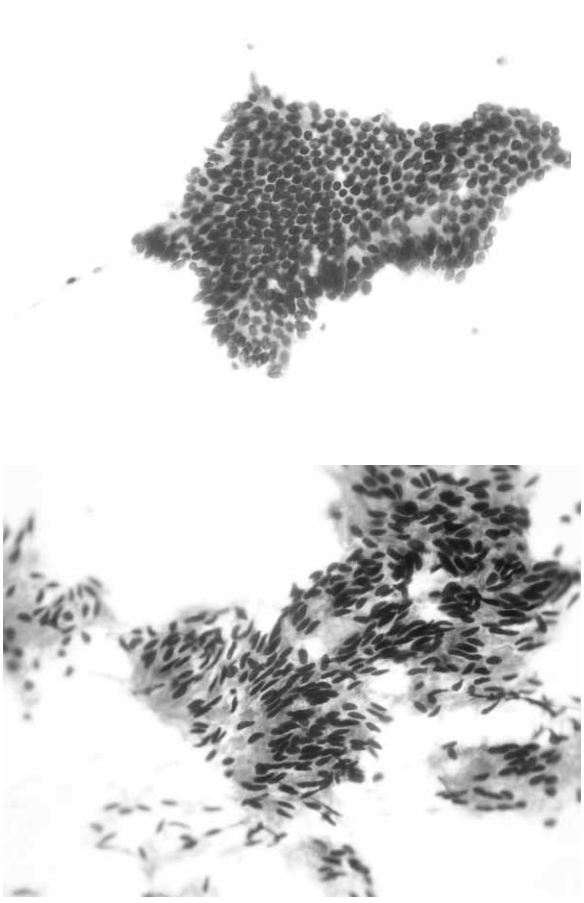

Fig. 3. Normal bile duct brushings a) group of benign epithelial cells; b) benign spindle cells (after sphincterotomy) (Papanicolaou stain, x1000).

smears (1%) due to scant cellularity[7,12]. Immediate ethanol fixation and air-drying of the rest of the slides enabled us to minimize impact of technical errors and air-drying artifacts on cytologic interpretation which some authors find to be important reason for false negative diagnoses[7]. We prefer to use two different methods of staining for evaluation of biliary brush specimens. Each staining has its advantages. Nuclear details are better appreciated on Papanicolaou stained smears but MGG staining lessen the impact of air-drying artifacts. The importance of clinical information and its impact on cytologic diagnosis has been documented on several occasions[8,9,17].

Greatest impact on biliary brushings sensitivity had a fact that 20 out of 143 (14%) brushings that had benign cytology were found to be malignant by other diagnostic methods or final clinical diagnosis. We reviewed all cases of false negative diagnosis and found that in this group most smears were not representative of the lesion, because the brush did not reach the malignant lesion. There was only one true false-negative where we initially missed malignant cells on the slides. On revision we found one cluster of malignant cells that was overlooked amongst clusters of otherwise atypical but reactive look-

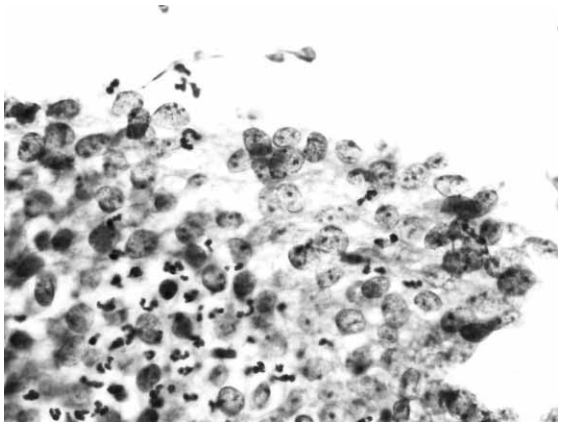

Fig. 4. Reactive changes (Papanicolaou stain, x1000).

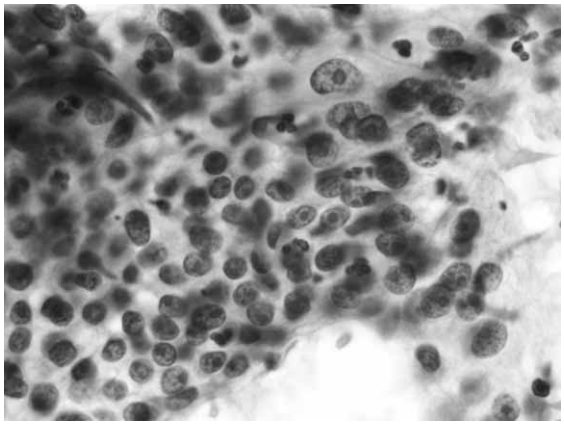

Fig. 5. Malignant cells (Papanicolaou stain, x1000).

ing epithelium. All other specimens had no malignant cells on slides even when careful searching was conducted, so we concluded that sampling was the most important factor that influenced cytological diagnosis. This finding is in agreement with most studies dealing with biliary brush cytology. It is worth mentioning that among those 20 cases there were 4 cases of gallbladder carcinoma, 11 cases of pancreatic cancer, only two cholangiocarcinoma of common bile duct and one primary site remained unknown but was presumably of pancreatobiliary origin. Two cases of ampullary carcinoma remained undiagnosed by cytology. With the exception of ampullary carcinoma, all other lesions were not easy to reach during the ERCP, but were diagnosed by other means including EUS guided FNA.

Excluding suspicious findings from analysis, there were no false-positive cases in this series, so our results for specificity were high as expected. One case classified as suspicious had diagnosis of high grade dysplasia on biopsy specimen from the papilla and when encountered in statistical analysis it caused the drop of specificity to 98.7%. Regarding other tumor types, cytology recognized and subsequently proved by immunocytochemistry one case of malignant melanoma metastatic to pancreas with malignant cells found in brushings, and there was also one case of non-Hodgkin lymphoma found. Accurate diagnosis had certainly influenced clinical management of those patients. We encountered two cases suspicious for mucinous neoplasm, probably malignant, but since both patients were from other institutions we were not able to trace relevant data regarding clinical follow up, so these cases were excluded from analysis. Cytologist interpretating brushing specimens should be familiar with cells types that are normally found in this type of material (Figures 3a and b) as well as with changes encountered due to technical reasons and reactive changes. Interpretation of inflammatory and reactive epithelial changes is always difficult, especially when atypical cells are but a few on the slide[17,23]. Number of benign conditions including inflammatory disorders, presence of stones, or stent placement may cause severe nuclear and sometimes architectural changes making it difficult to distinguish from malignant cells (Figures 4 and 5), thus raising a possibility of making false positive diagnosis. Sticking to the established diagnostic criteria[9,14] and refraining of making the diagnosis on technically less than satisfactory slides should lessen although would not completely solve the problem[24,25].

Number of ancillary techniques were evaluated in an attempt to achieve better sensitivity of biliary brush specimens for diagnosis of malignancy, including fluorescence in situ hybridization (FISH), digital image analysis (DIA) and molecular mutational studies[26–28]. Although they claim to increase diagnostic sensitivity, these methods are fairly expansive, need abundant material to be performed and are quite technically complex[17,18], so their introducing in routine practice is not only difficult at the moment[19] but perhaps also unnecessary. We find that biliary brush cytology, although mainly depending on the skill of endoscopist, as well as the experience of the cytologist, is a valuable method for obtaining accurate tissue diagnosis of biliary strictures thus solving eternal diagnostic dilemma: benign or malignant.

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CITOLOŠKI RAZMAZI BRISA ČETKICOM U DIJAGNOSTICI MALIGNIH PROMJENA BILIJARNOG STABLA: NAŠE ISKUSTVO

S A Ž E T A K

Preoperativno razlikovanje malignih od benignih suženja bilijarnog stabla usprkos novim dijagnostičkim mogućnostima još uvijek predstavlja izazov, a važnost tkivne dijagnoze je neupitna za izbor odgovarajućeg postupka liječenja. Razmaz brisa četkicom pri endoskopskoj retrogradnoj kolangiopankreatografiji (ERCP) i citološka analiza uzorka je danas uobičajena metoda za dobivanje tkivne dijagnoze. Dosadašnje studije su pokazale da je metoda visoko specifična ali uglavnom slabo osjetljiva zbog velikog broja lažno negativnih citoloških dijagnoza. Cilj ovog rada je bio provjeriti osjetljivost i specifičnost metode u našoj ustanovi, pronaći glavne uzroke lažno negativnih citoloških nalaza te potvrditi utjecaj timskog pristupa na citološku dijagnozu. Zlatni standard za dijagnozu bio je histološki nalaz ili klinički ishod utvrđen praćenjem najmanje šest mjeseci. Svi su razmazi napravljeni metodom direktnog nanošnja materijala sa četkice na staklo odmah po uzimanju uzorka u sobi za endoskopiju uz prisustvo citotehničara. Dio razmaza je odmah fiksiran u etanolu i bojan po Papanicolaou a dio sušen na zraku i bojan metodom po May-Grünwald Giemsi (MGG). Razmazi su rutinski analizirani i ocijenjeni kao benigni, atipični/reaktivni, suspektni i maligni. Od 143 uzorka s poznatim konačnim ishodom njih 36 (25%) je citološki ocijenjeno malignim, 91 (63.6%) uzorak je bio benigan, uz 3 atipična i 13 suspektnih. Konačna maligna dijagnoza je postavljena u 20 od 91 citološki benignog uzorka. Isključivši uzorke s atipičnim i suspektnim citološkim nalazom iz analize, osjetljivost metode iznosi 64% uz uobičajeno visoku specifičnost od 100%. Stavivši suspektne i pozitivne citološke nalaze u jednu kategoriju kako je uobičajeno, osjetljivost je narasla na 71%. Iako ovisi vještini endoskopičara i iskustvu citologa možemo slobodno zaključiti da je citološka analiza razmaza brisa četkicom pri ERCP-u vrijedna metoda za dobivanje pouzdane tkivne dijagnoze.