

## Endoscopic biliary tract brush cytology in 54 cases

Işın SOYUER<sup>1</sup>, Arzu TAŞDEMİR<sup>1</sup>, Şebnem GÜRSOY<sup>2</sup>, Alper YURCİ<sup>2</sup>, Ertuğrul MAVİLİ<sup>3</sup>, Nevzat ÖZCAN<sup>3</sup>

**Aim:** Duct brushing cytology is an important tool in the evaluation of the extrahepatic biliary tract. New liquid-based preparations and ancillary tests have emerged with the intent of addressing this issue.

**Materials and methods:** Fifty-four consecutive patients and 68 specimens with a suspected malignant obstruction of the common bile duct were included in a consecutive, nonrandomized order. We selected patients whose follow-up, in the form of either histology (25/54, 46%) or at least 6 months of clinical observation, was available. They underwent sampling during ERCP (33 patients/40 materials) or PTC (21 patients/28 materials) using the brush method.

**Results:** A total of 68 specimens were identified from 54 patients. The cytologic findings were: 35% benign, 37% malignant, and 9% suspicious for malignancy. Overall operating characteristics were: 48% sensitivity, 100% specificity, 100% positive predictive value, 69% negative predictive value, and 75% accuracy. For the diagnosis of malignant stenosis, the sensitivity was 48% for cytology, 60% for biopsies, and 50% for the combination of cytology and biopsies. In addition, the Cytospin method showed more cellularity compared with the other preparation method (n = 68 materials; 57% Cytospin, 38% direct smear).

**Conclusion:** Diagnosis of malignant biliary stenosis may be improved by a combination of endobiliary sampling and cytology.

**Key words:** Extrahepatic biliary tract, liquid-based cytology

### Endoskopik safra yolları fırçalama sitolojisi; 54 olgu

**Amaç:** Ekstra hepatik safra yollarının incelenmesinde safra duktus fırçalama sitolojisi önemli bir araçtır. Bu yöntemin yüksek orandaki spesifitesine karşılık maligniteyi saptamada sensitivitesi düşüktür. Yeni sıvı bazlı yöntemlerin kullanımı ve yardımcı teknikler bu konuda umut vericidir.

**Yöntem ve gereç:** Safra duktuslarında maligniteye bağlı daralma şüphesi olan 54 hastaya ait 68 materyal incelendi. Bu hastalardan biyopsisi olanlar (25/54, % 46) ve en az 6 ay klinik izlemi olanlar çalışmaya alındı. Bu hastalara ERCP (33 hasta/40 materyal) veya PTK (21 hasta/28 materyal) eşliğinde fırça sitolojisi uygulandı. Sonuç olarak 25/54 (% 46) hastada kanser tanısı [12/33 (% 36) ERCP ve 13/21 (% 61) PTK] verildi. Lamlar doğrudan yayma ve Sitospin yöntemi ile hazırlandı.

**Bulgular:** Toplam 54 hastaya ait 68 materyel değerlendirildi. Sitolojik tanılar: % 35 benign, % 37 malign, % 9 malignite şüphesi olarak sıralanmaktadır. Doğruluk oranları; % 48 sensitivite, % 100 spesifite, % 100 pozitif prediktif değer, % 69 negative prediktif değer ve % 75 doğruluk olarak bulundu. Malignite tanısı için sitolojinin sensitivite oranı % 48, biyopsinin % 60, her iki yöntemin birlikte kullanılmasında ise % 50 bulundu. Ek olarak Sitospin yönteminde preparatlar diğer yöntemden daha hücresel bulundu (n = 68 materyel; % 57 Sitospin, % 38 doğrudan yayma).

**Sonuç:** Malign safra yolu darlıklarının araştırılmasında endobilyer örnekleme tekniği zordur ve hastalığın saptanma oranı düşüktür. Biyopsi ve sitolojinin birlikte kullanımı ile doğruluk oranı artırılabilir.

**Anahtar sözcükler:** Ekstrahepatik safra yolları, sıvı bazlı inceleme

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<sup>1</sup> Department of Pathology, Faculty of Medicine, Erciyes University, Kayseri - TURKEY

<sup>2</sup> Department of Gastroenterology, Faculty of Medicine, Erciyes University, Kayseri - TURKEY

<sup>3</sup> Department of Radiology, Faculty of Medicine, Erciyes University, Kayseri - TURKEY

**Correspondence:** Işın SOYUER, Department of Pathology, Faculty of Medicine, Erciyes University, Kayseri - TURKEY

E-mail: isinsoy@erciyes.edu.tr

**Introduction**

Bile aspiration during endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic cholangiography (PTC) has been used as a diagnostic tool in the evaluation of pancreatic and biliary tract strictures for the last 2 decades. Both procedures allow transformation of a diagnostic study to a variety of therapeutic measures, including biliary drainage, removal of stones, placement of a stent, and dilation of a stricture. The sensitivity of brush cytology alone for the diagnosis of all malignant biliary strictures ranges from 33% to 58% (1-10). The popular combination of brush cytology and forceps biopsy has a sensitivity ranging from 52% to 70.4% (2-5). The recent growth and acceptance of liquid-based cytologic preparation methods has led to a more widespread use of thin-layer technologies (6,7).

In the current study, our aim was to review our institution's experience with duct brushing cytology over the past 2 years in order to determine operating characteristics of the procedure. In addition, we compared the efficacy of the usual direct brush smears to Cytospin technologies.

**Materials and methods**

**Patients**

The subjects were 54 patients with biliary stricture who underwent bile duct brushing cytology and/or biopsy between January 2006 and December 2007. In 33 patients, brush cytology was performed after ERCP. Twenty-one patients with biliary stricture underwent PTC. The series, including 40 men and 14 women with a mean age of 54 (range: 43-92), underwent endoscopic biliary brushing.

**Cytopathologic examination**

*Preparation and assessment of brush cytology specimens*

Two samples from each specimen were prepared. One was directly smeared, air-dried, and stained with May-Grünwald-Giemsa (Merck, Germany) before it was sealed with a cover slip. The brush was vigorously shaken in Cytospin collection fluid (Shandon, UK; green solution) to release a maximum of cells. One to two milliliters of fluid was used to prepare

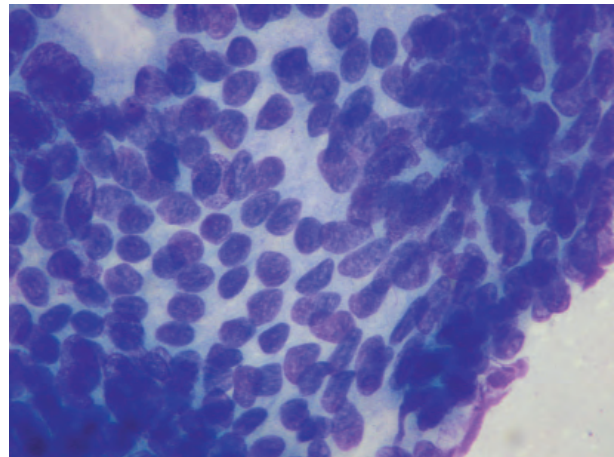
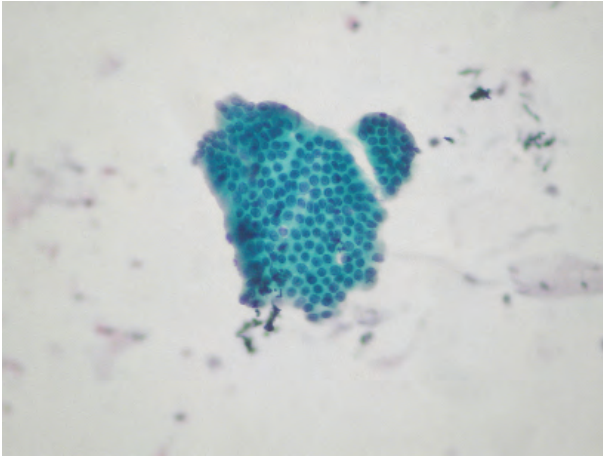
Megafunnel (Shandon, UK) on a cytocentrifuge (Cytospin 4, Shandon, UK). The samples from each specimen were spun down with 1300 cycles/min for 6 min. One glass was stained with Papanicolaou stain (Merck, Germany).

The global cellularity of each specimen was assessed in analogy with Camp's method (8). All specimens were graded and assembled in 4 groups, according to the number of epithelial cells per slide: grade 0, insufficient epithelial cells for interpretation (fewer than 5 clusters with  $\geq 10$  cells per cluster in  $\geq 2$  slides) (9); grade 1, low cellularity (<10% of the total slide area covered by epithelial cells); grade 2, moderate cellularity (10-40% of the total slide area covered by epithelial cells); and grade 3, high cellularity (>40% of the total slide area covered by epithelial cells) (Table 1).

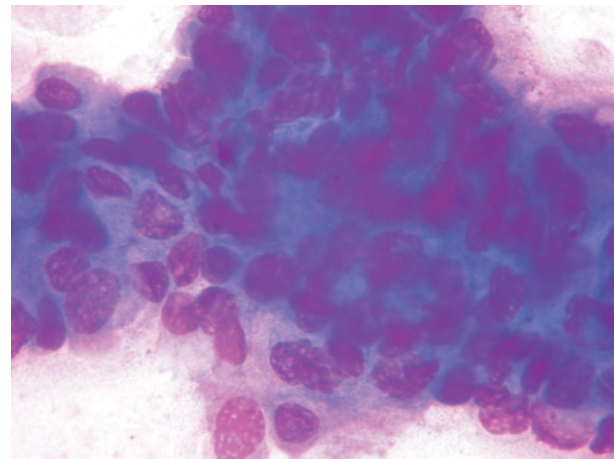
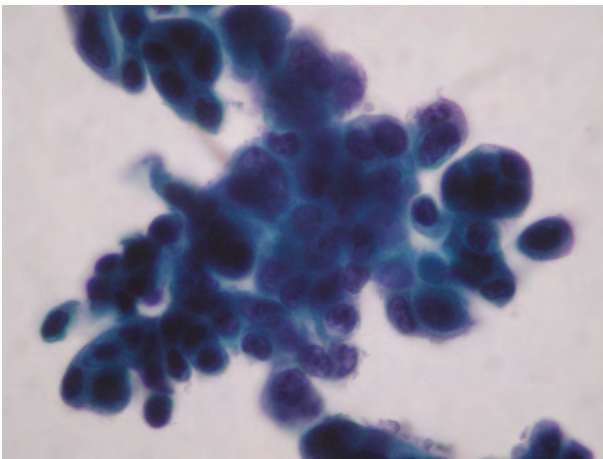
If abnormal cells were present, lower numbers of cells were accepted if the technical quality of the slide was good. We used generally accepted cytological criteria to classify the specimens into the following categories: benign, highly atypical/suspicious for cancer, or malignant. The final cytological classification for a patient was based on the most severe cytological finding. Representative photographs are shown in Figures 1-4. The interpretation of results reported as atypical was discussed among the authors, and it was agreed that, for the purpose of calculating sensitivity, specificity, positive/negative predictive values, and diagnostic accuracy, all diagnoses "highly atypical/suspicious for cancer" would be regarded as equivalent to cancer, and all diagnoses "atypical/considered reactive" would be regarded as benign.

Table 1. Comparison of cellularity between Cytospin and direct brush smear.

|                | Cytospin (n) | Direct brush smear (n) |
|----------------|--------------|------------------------|
| Grade 1        | 2            | 2                      |
| Grade 2        | 16           | 11                     |
| Grade 3        | 16           | 13                     |
| Grade 4        | 5            | 3                      |
| Total (n = 68) | 39 (57%)     | 29 (38%)               |



Figures 1 and 2. Benign biliary brush cytology (PAP ×200, MGG ×400).



Figures 3 and 4. Malign biliary brush cytology (PAP ×400, MGG ×400).

## Results

The material for cytology was sufficient for analysis in 54 cases and biopsies were obtained in 25 cases (46%). Each case had 1-4 biopsies, and our series contained 2 Whipple operation specimens (in total, 32 biopsy specimens). Sixteen patients underwent both bile duct cytology and biopsy. For the diagnosis of malignant stenosis, the sensitivity was 48% for cytology, 60% for biopsies, and 50% for the combination of cytology and biopsies. Three cases had different diagnoses for the cytology and the biopsy. Each of these 3 cases had pancreatic carcinomas; one of them was correctly diagnosed by cytology while the rest were correctly diagnosed by biopsy (Table 2).

ERCP had a higher sensitivity for the detection of cancer than PTC (7/12 [58%] versus 5/13 [38%]) and a higher diagnostic accuracy (21/26 [80%] versus 8/16 [50%]). Specificity for the detection of cancer was 100% (Tables 3 and 4).

## Discussion

Inflammatory processes, malignancy, and calculus disease may cause strictures of the extrahepatic biliary tract and pancreatic duct. Most benign strictures are managed conservatively with ductal dilatation and stenting. Malignant strictures may be treated by Whipple resection, bile duct resection, or simple stenting if the patient's disease is unresectable. Recent

Table 2. Overall diagnostic accuracy results.

|                   | Sensitivity | Specificity | ppv         | npv         | accuracy   |
|-------------------|-------------|-------------|-------------|-------------|------------|
| Cytology          | 48 (12/25)  | 100 (29/29) | 100 (12/12) | 69 (29/42)  | 75 (41/54) |
| Biopsy            | 60 (9/15)   | 100 (17/17) | 100 (9/9)   | 100 (17/17) | 78 (25/32) |
| Biopsy + Cytology | 50 (3/6)    | 100 (10/10) | 100 (3/3)   | 100 (10/10) | 81(13/16)  |

Table 3. Cytologic diagnosis for 2 different methods.

|              | Sensitivity | Specificity | ppv         | npv        | accuracy   |
|--------------|-------------|-------------|-------------|------------|------------|
| ERCP         | 58 (7/12)   | 100 (21/21) | 100 (7/7)   | 80 (21/26) | 84 (28/33) |
| PTC          | 38 (5/13)   | 100 (8/8)   | 100 (5/5)   | 50 (8/16)  | 61 (13/21) |
| Both methods | 48 (12/25)  | 100 (29/29) | 100 (12/12) | 69 (29/42) | 75 (41/54) |

Table 4. Result of biliary cytology for the diagnosis of malignant biliary strictures.

| Patients (n) | Number of malignancies | Cholangiocarcinoma | Pancreatic cancer | Other metastatic tumor |
|--------------|------------------------|--------------------|-------------------|------------------------|
| ERCP (33)    | 12                     | 6 (50%)            | 6 (50%)           | 0                      |
| PTC (21)     | 13                     | 6 (46%)            | 3 (23%)           | 4 (30%)                |

advances in neoadjuvant chemo- and radiotherapeutic approaches underscore the importance of accurate preoperative diagnosis by noninvasive means. Biliary sampling for cytopathologic examination during ERCP is performed using a brush, but brush cytology is limited by a low (18-57%) sensitivity for the detection of cancer (2,3,5,9,12-15). In the present study, bile duct brushing cytology and/or forceps biopsy was performed in patients with biliary stricture with or without obstructive jaundice. Sample collection rates, overall diagnosis rate, the diagnosis rate of each disease, and the influence on subsequent endoscopic biliary brushing were investigated.

Duct lesion sampling may be hindered by tumor desmoplasia, submucosal location of neoplasms, and extrinsic tumors that compress the duct, leading to the impression of a primary stricture. It has been reported that combinations of needle biopsy, forceps biopsy, and brush cytology increased the performance of diagnosis. The results varied among different

combinations. The most common combination, forceps biopsy and brush cytology, achieved good outcomes (55%-73%) (1,2,5). In our study, the combination of brush cytology and forceps biopsy improved the accuracy (81%) when compared with the accuracy of the individual procedures, suggesting that the combination of 2 or more examinations is necessary to improve the diagnosis rate, as previously reported (16-19).

Interpretation of cytologic atypia may be difficult, especially in cytologic preparations such as air-drying artifacts, and cellular obscuring by clumping, necrosis, and inflammation. The recent growth and acceptance of liquid-based cytologic preparation methods has led to more widespread use of thin-layer technologies, but there is little in the literature comparing different preparation methods for duct brushing cytology. In a study aimed at reclassifying atypical bile duct brush cytology, Okonkwo et al. (6) found cytocentrifuge specimens to be of superior quality compared with direct, alcohol-fixed smears.



Okonkwo et al. (6) and Volmar et al. (7) compared differences in cytologic features of bile duct brushings prepared by direct brush smear with those prepared with Cytospin preparation. They showed a statistically significant increase in sensitivity and diagnostic accuracy in brush cytology when the combination of liquid-based technology and direct smears was used. This combination was superior to both direct smear with Cytospin and direct smear alone.

Brush cytology at our institution showed modest sensitivity but high specificity for malignancy. The significant number of suspicious and atypical diagnoses that were associated with malignant follow-up emphasizes that bile and pancreatic duct lesions are difficult to sample and difficult to interpret. Furthermore, the findings indicate that a suspicious

or atypical specimen does carry some weight of malignancy and should prompt additional investigation. For both pathologists and clinicians, it is imperative that cytologic findings be interpreted in light of clinical presentation, imaging findings, and serum studies. We found that the performance characteristics of brush cytology were significantly affected by the characteristics of the sampled lesion and by the cytologic preparation method. Specifically, our data suggest that the combination of direct brush smear with liquid-based technology provides more sensitivity and accuracy relative to either Cytospin or direct smears alone. In addition, transpapillary brush cytology and forceps biopsy could be performed in a short amount of time. The diagnosis rate was high, and the incidence of complication was low, having no influence on subsequent biliary drainage.

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