Endoscopic brush cytology from the biliary duct system is still valuable

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ABSTRACT

INTRODUCTION: The aim of this study was to define sensitivity, specificity and positive and negative predictive values of endoscopic retrograde cholangiopancreatography (ERCP) brush cytology from biliary strictures obtained over a period of 12 years in a county hospital in Denmark.

MATERIAL AND METHODS: Patients with cytology specimens identified by brushings of the bile duct, pancreatic duct and ampulla of Vater were included. The specimens were reported as unsatisfactory, normal, atypical, suspicious for malignancy or malignant. Our evaluation comprised 75 specimens. For the statistical analysis, an atypical cytology result was considered benign, and a suspicious result was considered malignant. The cytological diagnoses were compared with the final diagnoses which were established either by histopathology (surgery, biopsy or autopsy) or by at least one year of clinical follow-up.

RESULTS: Of the 75 specimens included, 40 were diagnosed as cytologically benign (35 normal and five atypical) and 35 as cytologically malignant (22 suspicious for malignancy and 13 malignant). Comparing the cytological diagnosis with the final diagnosis, we found 35 to be true positives, 22 to be true negatives, zero to be false positives and 18 to be false negatives. Of the five atypical specimens, four were false negatives. The operating characteristics were: 66% sensitivity, 100% specificity, 100% positive predictive value and 55% negative predictive value. The diagnostic accuracy was 76%.

DISCUSSION: Suspicion and malignant cytology are reliable with a specificity of 100%. In these cases, we recommend that the patients are considered for surgical or oncological treatment without further histological investigations.

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Biliary strictures can be caused by various inflammatory diseases and by benign or malignant bile duct tumours. Distinguishing between malignant and benign biliary strictures remains a challenge.

Lesions of the pancreatic and biliary duct systems, including the ampulla of Vater, can primarily be investigated using endoscopic retrograde cholangiopancreatography (ERCP)-directed brush cytology. More advanced investigations, such as cholangioscopy and endoscopic ultrasound, are only used in selected cases.

The aim of the present study was to determine the sensitivity, specificity and positive and negative predictive values of the primary investigation, ERCP brush cytology, from biliary strictures in which malignancy was suspected and in which there was sufficient material to obtain a cytological diagnosis.

MATERIAL AND METHODS

Patients with elevated bilirubin and an ultrasound that showed dilated bile ducts without a visible tumour had primary ERCP with brushing.

The specimens collected by brushing were smeared onto an average of four slides immediately upon removal from the endoscope (direct smear). The specimens were initially air-dried and later methanol-fixed and May-Grünwald-Giemsa-stained in the laboratory.

All specimens were reported as unsatisfactory, normal, atypical, suspicious for malignancy or malignant. A specimen was deemed unsatisfactory if no slide contained at least five groups of cells with ten cells per group [1, 2].

Normal ductal epithelium consists of cohesive, flat "honeycombed" sheets of ductal epithelial cells with small, uniform nuclei and a smooth nuclear membrane [1, 3-7]. Atypical, reactive cells may show nuclear atypia, but the cells remain cohesive and appear as a monolayer [1, 3, 6].

Malignant cells are larger with an increased nuclear cytoplasmic ratio, nuclear crowding/overlapping (moulding), anisonucleosis and three-dimensional cell clusters [1-7]. If some but not all of these criteria are fulfilled, the specimen was diagnosed as suspicious for malignancy (**Figure 1**).

We did not distinguish between the pathologists who read the slides, since every specimen was read by a senior specialist pathologists with expertise in gastrointestinal histopathology and cytology, either directly or as supervisor, and since the intra- and interobserver agreement in cytological evaluation of endobiliary brushings from bile duct strictures evaluated by specialist pathologists is generally good [8].

Patients with cytology specimens obtained by brushings of the bile duct, pancreatic duct and ampulla of Vater were collected between 1 January 1995 and 31 December 2006 and were included in the study.

The patients were found in the national pathology database by searches for the brush biopsies from the

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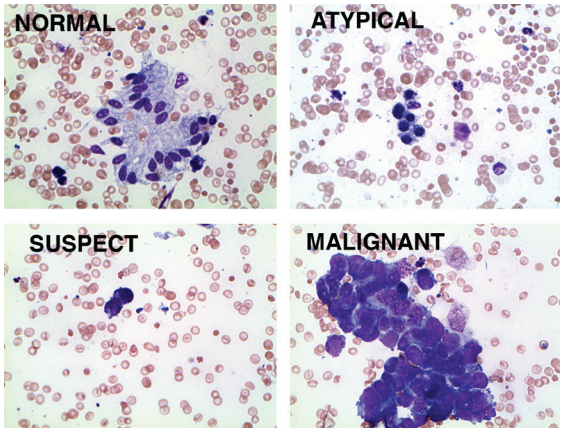
FIGURE 1

Normal ductal epithelium consists of cohesive, flat "honeycombed" sheets of cells with small, uniform nuclei and smooth nuclear membrane. Atypical, reactive cells may show nuclear atypia, but the cells remain cohesive and appear as a monolayer. Malignant cells are larger with increased nuclear cytoplasmic ratio, nuclear crowding/overlapping and three-dimensional cell clusters. If some but not all of these criteria are fulfilled, the specimen is diagnosed as suspect.



relevant localizations preformed at the local hospital during the period. At the Medical Department, brush biopsies were performed routinely in patients who underwent ERCP due to stenosis suspected for malignancy; that is in patients who had no history of pancreatitis or bile duct stone and no sign of this at ultrasound examination and whose biochemical values were elevated for bilirubin.

For the statistical analysis, an atypical cytology result was considered benign, and a suspicious result was considered malignant.

The cytological diagnoses were compared with the final diagnoses established by retrospective follow-up either by histopathology (surgery, biopsies or autopsy), information obtained from the National Danish Pathology Database in which all specimens examined by pathologists in Denmark are centrally registered, or by at least one year of clinical follow-up.

The follow-up involved review of patient records including feedback from other departments to which the patient was referred and – if the patient has died – the cause of death entered into the death certificates. If the patient had left Region Zealand this was impossible, and such cases were therefore excluded.

Patients lacking a registered diagnosis of malignancy within one year after the cytology procedure were considered to have a benign diagnosis, as malignancy was expected to have become clinically manifest by that time.

Trial registration: not relevant.

RESULTS

A total of 120 specimens from 109 patients were identified. In all, 30 patients had an inadequate clinical or pathological follow-up and were therefore excluded. Four patients had an unsatisfactory cytology and were also excluded.

Thus, our study comprised 75 patients (initially with a total of 80 specimens, but all the patients who repeatedly had tests ended up with the same cytological diagnosis, and so only one test per patient was calculated). The cytological findings were: normal 46.7% (n = 35), atypical 6.7% (n = 5), malignant 17.3% (n = 13), suspicious for malignancy 29.3% (n = 22) (**Table 1**).

A total of 75 patients with a median age of 64 years (range 30-89 years), of whom 47 (62.7%) were men, formed the basis of the statistical analysis.

In the statistical analysis, suspicious cytology was included as positive for malignancy and atypical cytology was included as benign. This yielded a total of 46.7% (n = 35) with a malignant cytological diagnosis and 53.3% (n = 40) with a benign cytological diagnosis.

Subsequent clinical follow-up (one year) or pathological data confirmed a benign diagnosis in 22 patients and a malignant diagnosis in 53 patients. Comparing the cytological diagnosis with the final one, we found 35 true positives, 22 true negatives, no false positives and 18 false negatives. Of the five atypical specimens, four were false negatives (**Table 2**).

The operating characteristics were: 66% sensitivity (95% confidence interval (CI): 51.7-78.5%), 100% specificity (95% CI: 84.5-100%), 100% positive predictive value and 55% negative predictive value. The diagnostic accuracy was 76%.

DISCUSSION

For the patients included in this study, no tumour was visible at ultrasound or computed tomography (CT). Fine needle aspiration and forceps biopsy guided by these types of imaging were therefore not possible in these patients [9]. Forceps biopsy and fine needle aspiration can be performed at ERCP, but brush cytology remains the simplest technique for obtaining tissue samples from biliary strictures at ERCP [9]. Although highly specific, the main limitation of brush cytology is its low sensitivity for the detection of pancreaticobiliary cancers. In most published studies, the diagnostic specificity is high, 80-100% [1, 7, 10, 11], while the sensitivity is in the 48-65% range [11-13]. Of the 30 patients who were excluded from this study because of inadequate follow-up, a third had a malignant cytological diagnosis, while half of the included patients had a malignant cytological diagnosis. If the last 30 patients had been included, maybe the result had been somewhat different.

In our study, the diagnostic sensitivity was 66%

TABLE 1

The cytological findings.

Cytological diagnosis	n (%)
Normal	35 (46.7)
Atypical	5 (6.7)
Suspect	22 (29.3)
Malignant	13 (17.3)

TABLE 2

Cytological diagnosis compared to follow-up. The values are n.

	Positive follow-up	Negative follow-up	Total
Cytological positives	35	0	35
Cytological negatives	18	22	40
Total	53	22	75

which, as previously mentioned, means that advanced investigation is needed if brush cytology is normal or atypical. On the other hand, we have shown that suspicious and malignant cytology are true with a specificity of 100%. [7, 11, 14]. When a patient with elevated bilirubin is diagnosed with a tumour in the extrahepatic bile ducts or duct choleductus, either by ultrasound or CT, he or she is immediately referred to a specialized surgical department. Patients in whom a tumour has not been demonstrated by ultrasound or CT constitute a diagnostic challenge. In these patients, an ERCP is the next step in the investigation, and the ERCP may be supplemented with a brush biopsy from a stenosis, if one is present.

The high rate of false negatives may be due to interpretation error, technical error and/or sampling error [12]. Interpretation error is failure to recognize well-differentiated tumours [12]. When a pathological diagnosis turns out to be a false positive an interpretation error has occurred, meaning that the pathologist has misinterpreted the cytological changes. In some studies, interpretation error can reach 7% [12]. One such error is more likely to occur when pathologists not specializing in gastrointestinal histopathology are providing the replies, or when the cytomorphological criteria mentioned above are not followed.

Sampling error is usually due to a difficult anatomic location of the lesion, procedure difficulties, significant fibrosis or ulceration [6, 12]. Especially fibrosis is important in this context, because it can make it difficult to obtain any cells during brushing, which will cause the specimens to be labelled as unsatisfied, and inability to make a cytological diagnose and fibrosis is probably the main reason for the exclusion of specimens in the present study. It has been reported that sensitivity is highest for cholangiocarcinoma, intermediate for pancreatic carcinoma and lowest in biliary obstruction due to metastatic disease, since metastatic disease and pancreatic cancer may be covered by an intact biliary mucosa [10, 15]. In our study, we did not classify the patients according to their final diagnosis.

The poor sensitivity of cytological examination for pancreatobiliary malignancy has recently been improved owing to the introduction of new ancillary cytological test methods, such as fluorescence in situ hybridization (FISH) [16] and polymerase chain amplification (PCR) [17, 18]. Especially in pancreatic cancer, interest centres on the tumour suppressor gene SMAD4/DPC4 (deletion in pancreatic cancer locus 4) and the oncogenic KRAS [19], both individually and together, as the presence of KRAS and/or absence of SMAD4 indicates malignancy [19, 20].

The overall conclusion is that bile and pancreatic duct brushing cytology showed a high specificity but a modest sensitivity of malignancy, so we propose that it should be considered to use these ancillary and more expensive tests for cases with a normal or atypical routine brush cytology.

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