The contribution of new methods in cytology for increasing sensitivity in the diagnosis of extrahepatic bile duct lesions

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The aim of this review is to provide a comprehensive analysis of the existing literature pertaining to cytology of extrahepatic bile ducts. A search using the keywords "biliary brush cytology" was conducted in the PubMed database, with a focus on recent articles. The inclusion criteria primarily encompassed publications addressing problematic biliary stenosis. Emphasis was placed on identifying articles that explored innovative or less-utilized examination techniques aimed at enhancing the sensitivity of cytological examination. This review presents a comprehensive overview of the various types of materials used in sampling and the corresponding sampling methods. Additionally, it explores cytological and cytogenetic techniques, such as fluorescence in situ hybridization (FISH) and genetic methods (miRNA, NGS, cfDNA). These techniques possess the potential to improve the accuracy of diagnosing bile duct tumors, although their sensitivity varies. Furthermore, their utilization can facilitate early therapy, which plays a crucial role in patient prognosis. Each examination is always dependent on the quality and quantity of material delivered. A higher sensitivity of these examinations can be achieved by combining biliary cytology and other complementary methods.

Key words: diagnosis of biliary strictures; cytology, fluorescence in situ hybridization; next generation sequencing; sensitivity

Received: March 21, 2023; Revised: September 26, 2023; Accepted: October 12, 2023; Available online: November 14, 2023
https://doi.org/10.5507/bp.2023.041
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INTRODUCTION

The pathology of the extrahepatic bile ducts is diverse. A common issue is stenosis or obstruction leading to the most striking clinical manifestations. Prognostically the most serious etiology includes malignant tumors, the incidence of which in the Czech Republic is higher than the world standard for both sexes, but roughly corresponds to European standards. In men, in long-term follow-up, it had stable 6,3; 6,4; 5,7; 6,2 cases per 100 thousand in the years 2016–2019, slightly decreasing in women. However, the incidence in women is higher than in men (9,1; 9,7; 8,6; 8,7 cases per 100 thousand in the years 2016–2019) (ref.[1-4]). Globally, however, the incidence of cholangiocellular carcinoma is highest in Asian regions, especially in Thailand (ref.[5-7]). The high incidence of cancer in this area is associated with infection by hepatobiliary flukes, which cause chronic inflammation and are considered carcinogens (ref.[8]). Biliary tract pathology is divided into three areas: intrahepatic, the region of the hepatic hilum, i.e. perihepatic, and distal, which also includes pathological lesions of the duodenal papilla. Clinically, they often manifest as stenosis. Histologically, it is a group of lesions arising mainly from the epithelial lining of the bile ducts, ranging from precancerous changes to cholangiocellular adenocarcinoma, which plays the most important role here[9]. We present an overview of these lesions in Table 1 (ref.[10]). The diagnosis of pathological changes in this region is further very difficult, due in particular to poor availability[11-13].

Diagnostic methods have grown considerably but the key methods and gold standard are endoscopic retrograde cholangiopancreatography (ERCP) and cytological examination of samples taken during this examination, in some cases supplemented by histological examination[14-16]. For the cytological assessment of the pancreatobiliary area, the Papanicolaou classification, has been established. This has 6 categories I.–VI., with the last, VI. indicating malignant cells (Table 2) (ref.[14,15]). Malignant cells are usually in the clumps of irregularly arranged ductal epithelial cells, often three-dimensional. Cell nuclei tend to be irregular in size and shape with coarse chromatin structure and an irregular karyomembrane[14,15].

Prompt and accurate diagnosis is of critical importance to these patients in terms of surgery and especially neoadjuvant therapy. A large proportion of these tumors in addition to post hepatic jaundice often manifest in an advanced, inoperable stage. It is equally important to avoid surgery for benign lesions that can be resolved endoscopically[16]. The incidence of cancer in this locality has been on the rise for the last 30 years and its prognosis is not good. For this reason, cytological examination of samples taken at ERCP is absolutely essential. However, according to a meta-analysis, Udayakumar Navaneethan

et al. found that the sensitivity and specificity of brush examinations in patients with bile duct malignancy is unfortunately relatively low at 45% (ref.[17]) and 99% (ref.[17]), respectively[17].

The list of methods cited in this review is in Table 3.

TYPES OF EXAMINED SAMPLES AND TECHNIQUES

During the ERCP, various types of samples are taken for cytological examination, namely bile aspirate, bile duct lavage and most often brush biopsy from the site of stenosis. In 2021, a paper was published in the Journal of International Medical Research that sought to find the causes of this low sensitivity. A total of 48 patients were examined, in 56.3% (ref.[18]) the cytological examination was positive, the sensitivity and specificity of this examination were 79.4% (ref.[18]) and 85.7% (ref.[18]), respectively. These results were compared with other parameters such as age of patients, sex, location of choledochal stenosis, bile duct wall thickness, maximal bile duct dilatation above the stenosis, number of cells in the cytological smear, CA19-9 and CEA levels. No association with any of these parameters was found[18]. Several studies have advocated 2–5 brush passes through bile duct stenosis to increase the sensitivity of brush cytology[19,20].

Aspirating stagnant bile in patients with obstructive bile duct stenosis can also be used for cytological examination. Mamta Gupta et al. compared the sensitivity and specificity of cytological examination of bile aspirate with the material obtained by brushing. The sensitivity of cytological examination of bile aspirate was 42.9% (ref.[21]), and specificity 100% (ref.[21]). The authors see the main disadvantage in the examination of bile aspirate as the low cellularity of the examined material[21]. In 2016, Fior-Gozlan et al. published an article on cytological examination of bile aspirate in patients with bile duct carcinoma and demonstrated that biliary aspiration cytology is a safe examination in patients with symptomatic biliary stenosis, and if this method was supplemented by brush cytology, the sensitivity of the cytological examination increased by up to 81% (ref.[22]). Another method demonstrably increasing the sensitivity of cytological examination using ERCP is the use of negative pressure in the brush cytology of stenoses. Malignancy was found in 31 of 44 (70.4%) (ref.[23]) compared to 22 of 88 (25%) (ref.[23]) without it[23].

The results of cytological examinations of bile aspirate sometimes differ quite significantly in publications. Our view, based on experience in routine practice, is that low yield is the result of a number of factors, starting with examination of the surrounding tissues, slow transport of the pathology department, low cellularity of the material and incorrect processing. The immediate transport of bile aspirate plays a key role here. The cells contained in this type of material rapidly cytolyze, which significantly affects and reduces the evaluability of the cytology specimen and correct diagnostics. Evaluation of the adequacy of the material can be made very quickly by the endoscopist when rapid on-site evaluation (ROSE) is used[24]. The cytological smear is immediately stained in the endoscopic room and assessed in the process by a cytopathologist. This increased adequacy in a singleinstitute study to 99% using ROSE after a mean of 2.6 passages. The diagnostic yield accuracy was 83% (ref.[24]), sensitivity 74.6% (ref.[24]) and specificity 98% (ref.[24]). The process of evaluation can be done online as well by scanning the smear. We have experience in this field at our institution and it is a good alternative.

A very relevant and informative study was published in 2015. The authors, Shinia Sugimoto et al. compared the sensitivity and specificity of cytological examination of bile from samples collected by different methods. In the case of bile aspirate, bile duct brushing and glass coatings were made. The brush was then rinsed with saline which was then used as the third type of material for cytologi-

Table 1. Primary epithelial lesions arrising in extrahepatic bile ducts according WHO Classification of tumours[6].

Primary epithelial lesions arrising in extrahepatic bile ducts
• Billiary intraepithelial neoplasia low grade
• Billiary intraepithelial neoplasia high grade
• Intraductal papillary neoplasm with low grade intraepithelial neoplasia
• Intraductal papillary neoplasm with high grade intraepithelial neoplasia
• Intraductal papillary neoplasm with associated invasive carcinoma
• Cholangiocarcinoma
• Squamous cell carcinoma NOS
• Adenosquamous carcinoma
• Carcinoma, undifferentiated NOS
• Neuroendocrine tumor NOS
• Neuroendocrine carcinoma NOS
• Mixed neuroendocrine-non-neuroendocrine neoplasm

Table 2. Papanicolau system for reporting pancreatobiliary cytology[10,11].

Category I: Nondiagnostic
Category II: Negative (for Malignancy)
Category III: Atypical
Category IV: Neoplastic: Benign
Serous cystadenoma
Neuroendocrine microadenoma
Lymphangioma
Category IV: Neoplastic: Other
IPMN or MCN (low/intermediate grade)
IPMN or MCN (high grade)
Neuroendocrine tumours
Solid-pseudopapillary neoplasm
Category V: Suspitious (for Malignancy)
Category VI: Positive or Malignant

IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm.

Table 3. Overview of methods.

Method	Patients	Sensitivity (%)	Specificity (%)	Limitations	Authors	Ref.
Bile aspirate	41	42.9	100	[b]	Gupta et al.	17
Bile aspirate	126	34	100	[b]	Sugimoto et al.	21
Bile duct lavage	126	70	100	[b]	Sugimoto et al.	
Brush cytology	126	32	100		Sugimoto et al.	
Brush rinsing	126	43	100	[b]	Sugimoto et al.	
Bile duct lavage	59	67.9	100	[b]	Motomura et al.	22
Biopsy + bile duct lavage	59	87.9	100		Motomura et al.	
Biopsy	59	67.9	100		Motomura et al.	
Biopsy	62	75	94		Tamanda et al.	24
Biopsy	25	44.4	100		Sugiyama et al.	25
Biopsy	28	31	100		Howell et al.	26
Brush with negative pressure	88	70.4	100		Abbasi et al.	19
Bile aspirate + brush cytology	239	81	100		Fior-Gozlan et al.	18
Brush cytology	730 [a]	45	99		Navaneethan et al.	13
EUS-FNA	3532 [a]	59	100		Garrow et al.	32
FISH with brush cytology	28	47–59	95	[c]	Bergquist et al.	34
FISH with brush cytology	76	89	97		Gonda et al.	40
FISH with brush cytology	90	63	100		Vlajnic et al.	45
FISH with brush cytology	35	53	100		Chaiteerakij	46
FISH with brush cytology	74	76	94		Dudley et al.	56
FISH with brush cytology	102	50.7	74.1		Zoundjiekpon et al.	48
ERCP extended by cholangioscopy	97	100	86.8		Fukuda et al.	27
ERCP extended by cholangioscopy	87	92.1	100		Osanai et al.	29
Cholangioscopy for direct visual examination	33	100	91.7		Nishikawa et al.	28
Cholangioscopy for direct visual examination	35	71	100		Chen	30
Brush cytology + ROSE	206	74.6	98	[d]	Archibugi et al.	20
Cytology, methylation markers and CA 19-9	67	97.4	92.4		Prachayakul et al.	67
CEA, CA19-9 and miR-1246	119	70.2	90.8		Ueta et al.	65
miRNA with brush cytology	73	92.9	100		Le et al.	64
NGS with brush cytology	94	93	100		Harbhajanka	49
NGS with brush cytology	74	85	98		Dudley et al.	56

ERCP, endoscopic retrograde cholangiopancreatography; EUS, endoscopic ultrasonography; FISH, fluorescent in situ hybridization; FNA, fine needle aspirate; n.a., not available; NGS, next generation sequencing; [a], metaanalysis; [b], low cellularity; [c], chromosomal aberations in patient with primary sclerosing cholangitis (PSC); [d], ROSE – rapid on-site evaluation.

cal examination, and lastly the lavage of the bile ducts as a fourth type of sample. All four sample types collected at ERCP were processed in the laboratory and evaluated by an experienced cytolpathologist. Papanicolau's classification was not used for evaluation but the results were classified as cat. I – benign, II – atypia, III – suspected of malignancy, IV – highly suspicious of malignancy and V – malignant. A total of 76 patients with cancer and 50 patients with benign bile duct stenoses were examined. The sensitivity of extrahepatic bile duct carcinoma was 34% (ref.[25]) for aspirate, 32% (ref.[25]) for brush smears, 43% (ref.[25]) for brush rinsing material and 70% (ref.[25]) for bile duct lavage performed after brush sampling[25].

In 2017, a paper was published in Gastroenterology, Hepatology and Endoscopy. The authors, Yasuaki Motomura et al. examined 59 patients, 40 of whom were assessed by bile duct biopsy taken at ERCP. They compared the sensitivity, specificity and diagnostic accuracy of bile duct lavage, biopsy and their combinations, ie lavage and biopsy. Biopsy sensitivity in this study was reported as 67.9%, 67.9% and 87.9% (ref.[26]), specificity 100%, 100% and 100% (ref.[26]), and diagnostic accuracy of 75.0%, 75.0% and 90.0% (ref.[26]), respectively.

Another attempt to improve the collection of material to ensure a larger amount of sample is modification of the collection tools themselves, especially the brushes. One study compared the standard brush used for ERCP sampling with a new type of brush. The authors showed a significant increase in the number of cell clusters per 20 fields of view using the new type of brush (Fig. 1) (ref.[27]).

Taking a biopsy sample from a bile duct stenosis can significantly help in the diagnosis of malignancy. Unlike brush cytology, it is a more technically demanding examination, dependent not only on the manual skill of the endoscopist and technical equipment but also on the width of the bile duct. In some cases, sphincterotomy is necessary. The sensitivity of forceps biopsy ranges from 31–75% (ref.[28-30]). The number of samples taken also plays

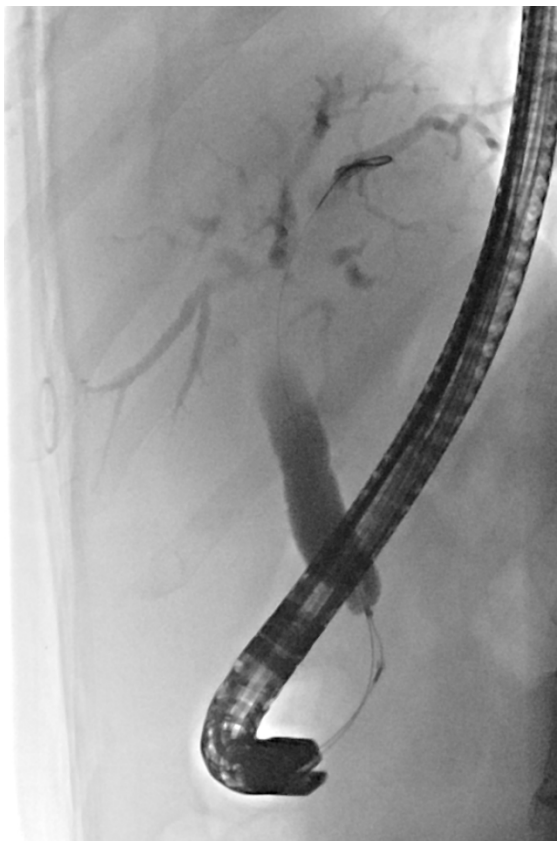

Fig. 1. Sampling by the brush method from the distal choledochus.

a role here. Tamada et al. found that three samples were sufficient for the diagnosis of intraductal papillary neoplasia associated with biliary carcinoma to achieve 100% sensitivity for the infiltrative type of tumour but multiple biopsies are necessary[28-30].

ERCP extended by cholangioscopic examination also contributed to the increase in sensitivity to 96–100% (ref.[31-33]). Currently, the digital single-operator cholangioscope is the technological standard of endoscopy departments (ref.[31-33]). The sensitivity and specificity for the visualization of bile duct damage was 100% (ref.[34]) and 91.7% (ref.[34]) in the diagnosis of malignant disease of the bile duct, respectively. The sensitivity and specificity in case of biopsy was 38.1% (ref.[34]) and 100% (ref.[34]), respectively.

In distal bile duct stenoses caused by carcinoma of the head of the pancreas, endoscopic ultrasonography (EUS) is used to visualize the tumor (Fig. 2), mostly as a hypoechoic or heteroechogenic focus, which can precisely aim the sampling needle. This greatly increases the sensitivity of the examination. The sensitivity of this examination without fine-needle aspiration of the sample was 78% (ref.[35]) and the specificity was 84% (ref.[35]). Today's standard EUS-FNA linear endoscopes enable endoscopic ultrasonography and fine-needle aspiration from the tumor area at the same time. DeWitt et al. published a meta-analysis in 2006 in patients with negative brush cytology. The sensitivity was 59% (ref.[36]) and the specificity was 100% (ref.[36]) using EUS-FNA (ref.[36]).

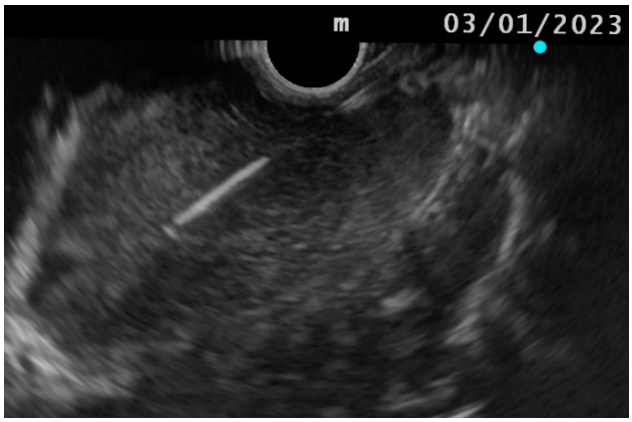

Fig. 2. EUS-FNB and sample collection from a suspected mass in the area of the head of the pancreas, which is stenosing the bile duct.

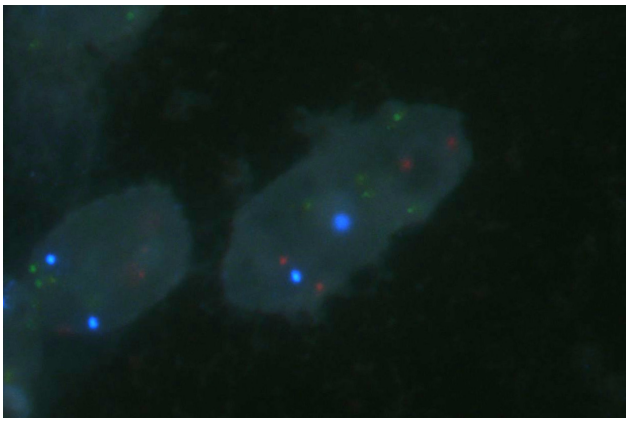

Fig. 3. Fluorescent in situ hybridisation: Small nonsuspitious nucleus and a large nucleus of an atypical cell with an increased number of green and red signals and a marked absence of yellow signals.

CYTOGENETIC METHODS

Efforts to improve the diagnosis of bile duct cancer have led to the gradual introduction of other examination methods, especially cytogenetic. In 2000, a study was published using flow cytometry to show a higher prevalence of cell aneuploidy in patients with primary sclerosing cholanigiitis and bile duct carcinoma compared to patients without cancer[37]. These findings have led to the development of cytogenetic examination of brush cytology by fluorescence in situ hybridization (FISH) using fluorescence probes binding to specific nucleic acid sequences and that detect chromosomal abnormalities (polysomy and aneuploidities) (Fig. 3). Today, it serves as a complementary examination method for more accurate diagnosis of cancer of the extrahepatic bile ducts. The most common changes in the genome of cholangiocellular carcinoma cancer cells have been described by researchers at the Mayoclinic. They found that the most reliable changes for detecting this cancer are polysomy of chromosomes 3, 7 and 17. In one study, they showed that the FISH method in combination with the classic cytological examination increases the sensitivity to 47–59%

(ref.[38]), while the specificity was 95% (ref.[38]). Very similar results are reported in cytology combined with FISH and elevated CA19-9 (ref.[39]). The problem is in patients with primary sclerosing cholangitis (PSC), in whom chromosomal abnormalities have also been detected. When trisomy of any of the chromosomes in bile duct cells was detected, the sensitivity increased but the specificity of the examination decreased. This was 93–95% (ref.[40]) in patients without PSC, and ranged from 70–87% (ref.[40]) in patients with PCS. Therefore, some of these patients were false positive. Later, high risk of developing cholangiocellular carcinoma in patients with PSC and cytogenetic changes was demonstrated[40]. Cholangiocellular carcinoma (CCA) in the PSC field is evidenced by more frequent MYC gene abnormalities and CDKN2A losses as well as higher clonal diversity but these changes, although increasing the sensitivity of the cytology, do not increase the specificity. However, changes in the MYC gene are more commonly observed in CCA in the PSC field than in primary CCAs, arising without PCS (ref.[41-43]). In 2012, Gonda et al. extended the detection of cytogenetic changes in bile duct stenoses and, in addition to the polysomy of chromosomes 3, 7 and 17, added the detection of a deletion of the 9p21 (p16) gene, which is a suspensor gene and is involved in cell cycle regulation[44]. The UroVision probe contains 4 color probes for labeling chromosome 3, 7 and 17 centromeres and the 9p21 (p16) gene and is used for examination in urogenital tract cytology as well, to increase the sensitivity of urinary cytology in an early diagnosis of urothelium carcinoma[44-48]. An increase in the number of signals above 5 in two or more chromosomes, in at least 5 cells, was determined as a positive result of FISH, ie polysomy. For the p16 deletion, the cut-off was set at a minimum of 10 cells in the case of a homozygous deletion and at least 6% (ref.[44]) of the cells in the sample in the case of a heterozygous deletion. The result was an increase in the sensitivity of the test from 21% (ref.[44]) in brush cytology to 58% (ref.[44]) on polysomy and further to 89% (ref.[44]) when the p16 deletion was included in the test (ref.[44]). The increase in the sensitivity of conventional cytological examination in combination with FISH to 63% (ref.[49]) was published by Vlajnic et al. in 2014 in Cancer Cytopathology[49]. A similar study comparing conventional cytology and FISH-supplemented cytology in patients of Asian origin was published in 2016. The sensitivity in conventional cytological examination increased from 33% (ref.[50]) to 53% (ref.[50]) in combination with FISH (ref.[50]). In 2015, Mayoclinic scientists optimized a set of probes for FISH examination of bile duct stenoses and they demonstrated a higher sensitivity than the UroVision set[51]. The above results are consistent with our experience. The results of our published work included 102 patients with bile duct stenosis of unknown etiology who underwent ERCP and were sampled for routine brush cytology as well as FISH. The sensitivity of the combination of brush cytology and FISH was 50.7% (ref.[52]) with a specificity of 74.1% (ref.[52]), while for brush cytology it was only 36.1% (ref.[52]) with a specificity of 85.2% (ref.[52]). The results of the sensitivity of the combination of these examinations were lower in our study than, Gonda et al. The difference is that our work focused on all patients with stenosis of the extrahepatic bile ducts. Some patients with extrinsic stricture had negative cytological and FISH examinations, which affected the results. There were clear differences in patients with intrinsic stricture, where the sensitivity and specificity were 69.2% (ref.[52]) and 73.3% (ref.[52]). In the group with extrinsic stricture, the sensitivity and specificity were 41% (ref.[52]) and 75% (ref.[52]). These cytogenetic changes will most likely not play a role in the cytological diagnosis of carcinomas in the pancreatobiliary region, but they may have prognostic or therapeutic significance in the future.

In addition to the already mentioned cytogenetic changes, which according to the published works increase the sensitivity of cytological examination, a number of works have been published that deal with genomic alterations of cholangiocellular carcinoma depending on its localization. One of them is the work of Zheng et al. published in 2021. The authors examined 450 genes in 270 patients with cholangiocellular carcinoma. They demonstrated differences in the mutations of some genes in different cancer locations. The work compared both intrahepatic and extrahepatic localized cancer, where it demonstrated an increased mutation of TP53, SMAD4 and ERBB2 genes in extrahepatic localization of cancer, while in the case of intrahepatic cancer there was a significant difference, especially in the case of IDH1, FGFR2, BAB1 and PBRM1. Similar changes were also found in the case of a comparison of perihilar and distally localized carcinoma, where TP53 and KRAS mutations dominated in the case of distal carcinoma, while PIK3CA, FAT4, MDM2 and TCF2L2 occurred mainly in tumors in perihilar localization[53.]

NEXT GENERATION SEQUENCING

Currently, sequencing and mutation analysis using next generation sequencing (NGS)s appears to be very promising in the diagnosis of bile duct lesions. This technology allows fast simultaneous sequencing of genetic material in a single process. Harbhajanka et al. found that the combination of cytomorphological analysis with molecular profiling using NGS led to a significant increase in the sensitivity of the examination from the original 49% (ref.[54]) to 93% (ref.[54]), especially in the case of malignant lesions. The most frequently identified gene alterations in malignancies were KRAS and TP53. Other more common alterations affected the CDKN2A, PIK3CA, SMAD4, ERBB2 genes, and almost half showed two or more gene alterations regardless of the type of malignancy[54]. These genes, as well as B2M, BRAF, CCND1, CTNNB1, FBXW7, NF1, PTEN and U2AF1, are captured in pancreatic adenocarcinoma in agreement with other authors[55-57]. In the case of cholangiocarcinoma from extrahepatic pathways, similar alterations were found in Javle et al. with the highest proportion of KRAS (42%), TP53 (40%), CDKN2A/B (17%) and SMAD4 (21%) (ref.[58]). A relatively recent review from 2021 describes a number of clinical studies underway[59]. Kushnir et al.,

showed that whether it is a combination of the FISH method or PCR-based mutation profiling (commercially available as PancraGEN, Interpace Diagnostics) with cytology, both combinations clearly lead to a higher detection of malignant lesions compared to cytology alone (44% and 56% vs. 26%) (ref.[60]), and a combination of all three 66% (ref.[60]). Similar results were compared combining cytology with FISH or NGS in Dudley JC. The increase in sensitivity was 76% (ref.[61]) and 85% (ref.[61]) respectively, compared to separate cytology, where the sensitivity was 67% (ref.[61]). NGS in malignancies has most often demonstrated driver mutations KRAS, TP53, SMAD4, CDKN2A. Each method has its advantages and disadvantages. The disadvantage of multicolour FISH is that it is more costly than NGS, and subtraction can be a challenge due to the nuclear overlap and therefore difficult copy counting. The advantage of multicolourFISH is lower cost than NGS, but subtraction can be a challenge due to the nuclear overlap and therefore difficult copy counting. In turn, it is important to interpret NGS results with respect to clinical correlation, especially in regard to KRAS, as KRAS mutations can also be detected in non-invasive lesions and some inflammatory conditions[61]. In 2017, Jusakul et al. published an interesting work in which they analyzed 489 cholangiocellular carcinomas from patients from 10 countries with endemic occurrence of infection caused by hepatobiliary flukes using NGS. The authors of the work demonstrated the differences between fluke-positive carcinomas in which ERBB2 amplification and TP53 gene mutation are frequent. On the other hand, fluke-negative carcinomas showed a high number of copies of PD-1/PD-L2 expression or epigenetic modifications of IDH1/2, BAP1 as well as FGFR/PRKA translocation. The work further pointed out that different anatomical locations of cancer do not determine the molecular subtype, tumors in different locations can show molecular similarities, but tumors in the same location can have significant differences. At the same time, they found that cholangiocellular carcinoma tumor survival trends did not differ in based on location, but molecular subtypes showed significant differences in survival[62]. In addition to basic mapping of biliary pathway mutations, NGS results are also therapeutically useful, especially in the case of molecularly tailored therapeutic strategies. Similar to tumors in other locations, there are already stratifications into therapeutically different groups with targeted therapy for different subtypes. A large part of the therapeutics is already used in other malignancies. Studies are currently underway that would enable their use in cholangiocellular carcinoma also. Some of them are already approved by the US Food and Drug Administration (FDA). These are mainly therapeutics aimed at mutations or rearrangements, especially FGFR2, then also IDH1, NTRK; V600E mutation of the BRAF gene (BRAFV600E); TMB-H/MSI-H/dMMR. Ongoing trials targeting HER2 and RET mutations are also promising[63-65].

MicroRNA DETECTION

MiRNA detection is another method that can be used in the diagnosis of malignant bile duct tumors to increase the sensitivity of cytological examination. This is based on the detection of miRNAs in brush or bile material. miRNAs are small non-coding RNA molecules approximately 21–28 base pairs in length whose function is to regulate mRNA expression. Their existence was first described by Lee et al. in 1993 (ref.[66]). Several papers have addressed this issue on their diagnostic potential for the detection of certain types of miRNAs in bile and attempt to establish a diagnostic miRNA panel for cholangiogenic carcinoma (ref.[67-72]). In 2019 Le et al. supplemented the conventional brush cytological examination of the bile ducts by detectionof miRNA expression (miR-16, miR-21, miR196a, miR-221) (ref.[73]), ie those in which dysregulation occurs most frequently in pancreatobiliary carcinoma cells. There were significant differences in expression of miR-16, miR-196a and miR-221 (ref.[73]) between samples of pancreatobiliary carcinoma and samples without a tumor. The best results were seen in the expression of miR-196a (ref.[73]), which the authors consider to be significantly diagnostic. When the conventional cytological examination and the expression of this miRNA were combined, the sensitivity of the examination of biliary stenoses was up to 92.9% (ref.[73]), with a specificity of 100% (ref.[73]). A very interesting study on the diagnosis of gallbladder and bile duct cancers was published in 2021. Ueta et al. proved that the combination of CEA, CA19-9 and miRNA-1246 (ref.[74]) detected from serum has high diagnostic potential with a sensitivity 72.0% (ref.[74]) and specificity 90.8% and miRNA-1246 (ref.[74]) was an independent prognostic marker. They also studied miRNA-451a (ref.[74]), which inhibited cell proliferation and induced apoptosis. The authors showed that miRNA-1246 and miRNA-451a detected in serum has the potential to be a diagnostic and prognostic marker and miR-451 (ref.[74]) a may be a novel therapeutic target[74].

OTHER METHODS

Another methodology leading to increased sensitivity of brushing examinations was used by Keane et al. 2017 using an immunocolorimetric ELISA test determining in this case "minichromosome maintenance replication protein 5" (MM5) (ref.[75]), important in the replication complex in initiating DNA synthesis. In the case of malignant strictures, the sensitivity increased to 55.6% (ref.[75]) compared to the original 25% in separate cytology[75]. A further increase in diagnostic sensitivity is possible by determining the methylation markers of DNA, especially in the area of promoters of some genes. In Prachayakul et al. differences between cholangiocellular carcinoma tumor cells and benign cells in the methylation index of Homeobox A1 (HOXA1) Neurogenin 1 (NEUROG1) genes were demonstrated. The combination of brush cytology, methylation markers and CA 19-9 increases the sensitivity and accuracy of the examination to 97.4% and

91.0% (ref.[76]). In addition, methylation markers were positive in 5 of 6 patients with confirmed cholangiocellular carcinoma who were cytology as well as CA19-9 negative[76].

In an attempt to diagnose early and reduce the mortality of patients with bile duct cancer, several studies using liquid biopsies and detection of tumor cell-free DNA (cfDNA) have been published in recent years. This is a method of detecting tumor extracellular DNA, which is released by the tumor into the blood and can therefore be used for the early detection of cholangiogenic carcinoma as well as tumors in other locations, for the detection of tumor heterogeneity[77,78]. Berchuck et al. published an extensive study in 2022 in which they evaluated samples from 1671 patients with advanced cholangiocellular carcinoma using NGS. Genetic changes in cfDNA were detected in 84% (ref.[79]) of patients. They found a relatively high agreement in the detection of mutations in cfDNA and in tumor tissue samples for IDH (87%) and BRAF (100%) (ref.[79]). In the case of fusion genes, detection was low, e.g. only 18% for FGFR2 (ref.[79]). They also demonstrated that a high frequency of variant cfDNA alleles is associated with a poor prognosis[79].

Lapitz et al. focused on the liquid biopsy used in patients with PSC and PSC and cholangiocellular carcinoma arising in the field of this chronic inflammatory disease. The authors defined extracellular vesicles that serve for mutual communication between cells and that contain a whole range of biomolecules such as proteins, nucleic acids, lipids, and metabolites. Using the ELISA method and a number of proteins that seem suitable for the early detection of cholangiocellular carcinoma[80].

CONCLUSION

The amount of material obtained from the endoscopic examination is very limited and the primary cytological examination itself, although specific, has only limited sensitivity. In the case of unclear results, other methods can be used, but additional material is needed. This must be available when the primary sample is taken. However, each examination has its limitations and even in the case of very sensitive methods such as NGS, there are false-negative and false-positive cases. However, the primary requirement is and that each examination is always dependent on the quality and quantity of material delivered. In the future several modern methods, together with basic and routinely used methods such as cytological examination during ERCP, will play an important role in the diagnosis of malignant diseases, not only in the pancreatobiliary area.

Search strategy and selection criteria

The aim of the work was to map the possibilities of increasing the sensitivity of the cytological examination of the extrahepatic bile ducts. The article is focused on an overview of the largest possible number of published methods studied for this purpose. We tried to include both clinical methods aimed at adequate tissue sampling, as well as methods supplementing cytological examinations, i.e. cytogenetic or genetic. The keywords cytology, bile duct stenosis, increased sensitivity was entered into the search engine. We tried to include especially publications from the last 10 years. Of course, the choice was also influenced by the experience of our clinical practice and the practice of a pathologist.

Acknowledgement: The study was supported in part by the Czech Ministry of Health (DRO: FNOL00098892), the Czech Ministry of Education (DRO: 61989592) and by Palacky University (LF_2023_003).

Author contributions: DK, ZS: participated in the creation of the text of the article almost equally and actively collaborated on all modifications; VDZ, OU: supplemented, according to their experience, the parts of the article concerning the collection of cytological material and the possibility of available endoscopic methods, including suitable references.

Conflict of interest statement: None declared.

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