Diagnostic approaches for cholangiocarcinoma

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Abstract
Cholangiocarcinomas arise from the epithelial cells of the bile ducts and are associated with poor prognosis. Despite new diagnostic approaches, the definite diagnosis of this malignancy continues to be challenging. Cholangiocarcinomas often grow longitudinally along the bile duct rather than in a radial direction. Thus, large tumor masses are frequently absent and imaging techniques, including ultrasound, CT, and MRI have only limited sensitivity. Tissue collection during endoscopic (ERCP) and/or percutaneous transhepatic (PTC) procedures are usually used to confirm a definitive diagnosis of cholangiocarcinoma. However, forceps biopsy and brush cytology provide positive results for malignancy in about only 50% of patients. Percutaneous and peroral cholangioscopy using fiber-optic techniques were therefore developed for direct visualization of the biliary tree, yielding additional information about endoscopic appearance and tumor extension, as well as a guided biopsy acquisition. Finally, endoscopic ultrasonography (EUS) complements endoscopic and percutaneous approaches and may provide a tissue diagnosis of tumors in the biliary region through fine-needle aspiration. In the future, new techniques allowing for early detection, including molecular markers, should be developed to improve the diagnostic sensitivity in this increasing tumor entity.

INTRODUCTION
Cholangiocarcinomas are topographically categorized as intrahepatic or extrahepatic carcinomas. Extrahepatic cholangiocarcinomas are further subdivided into hilar, middle and distal carcinomas. The most common type of hilar cholangiocarcinoma is classified into 4 stages according to the bismuth classification[1]. Surgery is the only curative treatment in patients with cholangiocarcinoma. The results are more favourable for patients with early-stage disease. Therefore, a reliable diagnostic procedure is of great importance for these patients. However, confirmation of cholangiocarcinoma can be very difficult because of a wide spectrum of alternative diagnoses, including other carcinomas, metastasis and benign biliary strictures. Therefore, multidisciplinary investigative approaches are needed to overcome this problem. Cholangiocarcinomas often grow longitudinally along the bile duct rather than in a radial direction away from the bile duct. Consequently, imaging techniques including ultrasound, CT, and MRI are of limited sensitivity for the detection of cholangiocarcinoma[2]. Biliary tissue collection during endoscopic procedures is widely used for distinction between benign and malignant strictures and provides the only definitive diagnosis that can be used for establishing therapeutic strategies. To obtain tissue samples, brush cytology and/or forceps biopsy were routinely performed in patients with suspected malignant biliary strictures.

BIOCHEMICAL INVESTIGATIONS
Obstructive jaundice is typically associated with an increase of serum bilirubin, alkaline phosphatase and gamma-
glutamyl transpeptidase. These laboratory parameters are unspecific and do not allow a distinction between benign and malignant bile duct strictures. The most widely studied tumor markers are carbohydrate antigen (CA) 19-9 and carcinoembryonic antigen (CEA). Both tumor markers may be elevated in cholangiocarcinoma[3-5]. However, CA19-9 and CEA are not specific for cholangiocarcinoma. CA19-9 is also raised in pancreatic cancer, colorectal cancer, gastric cancer, and gynaecological malignancies[6]. Additionally, CA19-9 may be elevated in patients with acute cholangitis[7]. In a series of patients without primary sclerosing cholangitis, the sensitivity of a serum CA19-9 level of more than 100 U/mL in diagnosing cholangiocarcinoma was 53%[8]. Furthermore, the authors reported in patients with unresectable cholangiocarcinoma a significantly greater mean CA19-9 concentration compared to patients with resectable cholangiocarcinoma. Recently, John et al[9] reported that sensitivity and specificity were 67.5% and 86.8%, respectively, when using a cut-off value of 100 U/mL. In another report that included 37 patients with primary sclerosing cholangitis, a serum CA19-9 concentration above 100 U/mL sensitivity was 89% and specificity was 86% for the diagnosis of cholangiocarcinoma[9]. CEA also has unsatisfactory diagnostic specificity and sensitivity for cholangiocarcinoma[9]. In conclusion, the diagnostic value of tumor markers in cholangiocarcinoma is limited. However, CA19-9 is useful in following the effect of treatment and to detect disease recurrence.

IMAGING

Ultrasoundography

Patients suffering from jaundice usually undergo transabdominal ultrasonography to evaluate the bile duct diameter and hepatic parenchyma. Furthermore, gallstones can be excluded. In most patients cholangiocarcinomas are not directly detectable, but indirect signs are visible in the majority of patients. Distal lesions cause dilation of both intrahepatic and extrahepatic bile ducts, whereas proximal lesions only cause dilation of intrahepatic bile ducts. The localization of the bile duct lesion can be suggested if there is an abrupt change in ductal diameter. The diagnostic accuracy of ultrasonography was investigated in 429 patients with obstructive jaundice. In this series ultrasonography demonstrated ductal obstruction in 89%, and the sensitivity for localizing the site of obstruction was 94%[10]. The sensitivity and specificity of ultrasonography depends on tumor localization, the quality of the equipment and the experience of the investigator[10]. Ultrasound findings are limited in patients with liver cirrhosis and primary sclerosing cholangitis due to a lack of visible dilated bile ducts. Doppler ultrasonography provides information on hepatic and portal vessel patency. Recent studies reported that contrast enhanced ultrasonography provides sensitive and specific criteria for the differentiation between malignant and benign liver lesions[12-15]. Preliminary data for cholangiocarcinoma suggest a behavior that is not dissimilar to metastatic lesions[14,16]. However, the limited number of cases in the reported series does not allow conclusive considerations for cholangiocarcinoma. Therefore, further studies with appropriate numbers of patients are needed.

Computed tomography

Computed tomography (CT) is a commonly used approach for the detection and staging of cholangiocarcinoma. The radiological findings depend on localization and morphology of the tumor. CT scan permits identification of bile duct dilatation as well as assessment of lymph node, liver parenchyma, vascular encasement and metastasis[17]. Additionally, computed tomography is useful for detecting the presence of liver atrophy. Dilatation of bile ducts combined with atrophy suggests the obstruction of the portal vein[18]. However, conventional computed tomography is limited in the ability to estimate the extent of cholangiocarcinoma and resectability. Tillich et al[19] reported a series of 29 patients with hilar cholangiocarcinoma who underwent multiphasic helical CT, including arterial and portal venous phase. In these patients resectability was correctly predicted in only 60%. In another series, Yamashita et al[19] reported only 59% sensitivity in identifying a primary lesion by using contrast-enhanced computed tomography. Recently, the accuracy of preoperative high-resolution computed tomography to determine resectability in patients with hilar cholangiocarcinoma was evaluated[20]. In this series negative and positive predictive values of high-resolution computed tomography to determine resectability were 92% and 85%, respectively. Thus, only new CT scanning techniques should be taken into account since radiological procedures have had a considerable improvement in the last years.

Magnetic resonance imaging and magnetic resonance cholangiopancreatography

In recent years, magnetic resonance imaging (MRI), especially in combination with magnetic resonance cholangiopancreatography (MRCP) has improved diagnosing cholangiocarcinoma and determining resectability[21-23]. Magnetic resonance imaging can assess the local tumor extension, lymph nodes, metastasis and liver parenchyma. It is important to use sequences with thin-slice thickness (3-4 mm) that provide sufficient signal to obtain good quality images and are sufficiently thin to detect subtle abnormalities. At present, good quality MRI in the hands of experienced centers, can be an excellent imaging approach for the diagnosis and staging of cholangiocarcinoma[24]. Moreover, magnetic resonance angiography (MRA) provides good assessment for infiltration of blood vessels. Magnetic resonance cholangiography can provide a three-dimensional reconstruction of the biliary tree without injection of intravenous and biliary contrast fluid. Therefore, the risk for cholangitis is reduced[21], and additionally there is no
risk for contrast induced nephropathy. MRCP allows the assessment of bile ducts above and below a total obstruction. Therefore, MRCP should be considered for planning the treatment of patients suffering from cholangiocarcinoma. Zidi et al reported a correct malignant hilar tumor stage using MRCP in 78% of the investigated patients. Furthermore, in this series an underestimated tumor extension was reported in 22%.[25] Biliary stent placement and percutaneous drainage results in mild inflammation of bile duct walls, which appears as an increased gadolinium enhancement with an appearance indistinguishable from the superficial spread of cholangiocarcinoma. To avoid this problem MRI and MRCP should be performed before endoscopic stenting and percutaneous transhepatic drainage.[23]

Positron emission tomography (PET)
Several studies reported intensive accumulation of nucleotide tracer 18-fluorodeoxyglucose (FDG) in cholangiocarcinoma.[26–28] PET scanning with focal FDG accumulation permits visualization of cholangiocarcinomas. PET scan can detect cholangiocarcinomas as small as 1 cm.[29,30] FDG-PET is of value for staging of bile duct cancers, especially for discovering distant metastasis and malignant lymph nodes. In one series, PET led to a change of therapeutic management in 30% of patients suffering from cholangiocarcinoma because of detection of primary unsuspected metastases.[22] The limitation of FDG-PET is false positive results in patients with biliary tract infections, primary sclerosing cholangitis, and biliary stenting via endoscopic retrograde cholangiography (ERC) and PTBD.[26,31] The diagnostic sensitivity can be increased by using 18-fluorodeoxyglucose (FDG) in combination with CT scanning (FDG-PET/CT). Reinhardt et al.[32] evaluated the effectiveness of this new dual-modality technique for noninvasive differentiation of extrahepatic bile duct strictures. This series included 14 patients with histological proven cholangiocarcinoma and 8 patients with benign bile duct strictures. In this series, all patients with cholangiocarcinoma presented with focal increased tracer uptake compared to patients with benign bile duct stricture. Overall, our experience is that 18F-FDG PET/CT does not provide high accuracy for noninvasive detection of perihilar cholangiocarcinoma in extrahepatic bile duct strictures, which may be mainly due to the small size of the tumors.

ENDOSCOPIC APPROACHES

Endoscopic retrograde cholangiography
Retrograde injection of contrast fluid into the biliary tract allows the assessment of localization and morphology of bile duct strictures. Malignancy is suggested when there are findings of asymmetric, irregular strictures. Moreover, resectability can be evaluated. However, the differentiation in benign and malignant bile duct stricture may be difficult. Park et al[23] identified 20 out of 27 malignant bile duct strictures using ERC alone. In this series diagnostic sensitivity and specificity for endoscopic retrograde cholangiography was 74% and 70%, respectively. Other authors have reported similar results for detecting malignant bile duct strictures by direct cholangiography.[33] Compared to non-invasive imaging techniques, endoscopic retrograde cholangiography allows tissue collection for cytological and histological investigation. Additionally, ERC allows biliary stent implantation for palliative treatment in irresectable tumors.

Percutaneous transhepatic cholangiography (PTC)
In patients with difficult bile duct access percutaneous transhepatic approaches offer a valuable alternative for bile duct access. The effectiveness of this procedure in diagnostic and therapy of complex biliary obstruction has been well documented.[34,35] Because percutaneous transhepatic bile duct access is an invasive technique, potential complications including bleeding, cholangitis, biliary leakage, duodenal perforation and death can occur. In previous series, procedure related death ranging from 0.6% to 5.6% was reported.[36–39] Therefore, endoscopic retrograde cholangiography is usually favoured above percutaneous transhepatic cholangiography. Percutaneous transhepatic approaches also allow tissue collection and biliary drainage.

Cholangioscopy
Cholangioscopy using fiber-optic techniques provide direct visualization of the biliary tree. Differentiation between benign and malignant bile duct stricture using a cholangioscope has not been well defined. However, typical signs for malignancy including mucosal ulcerations, irregular mucosa and asymmetric stricture may be visible. Moreover, cholangioscopic guided forceps biopsy and brush cytology may enhance the diagnostic accuracy of tissue diagnosis. The most common approach is percutaneous transhepatic cholangioscopy. Another possibility is to perform peroral transpapillary cholangioscopy using a mother baby endoscope. Fukuda et al.[40] evaluated the utility of peroral cholangioscopy for distinguishing malignant from benign biliary disease. The authors identified 22 out of 38 malignant bile duct strictures using ERC in combination with tissue sampling. The addition of peroral cholangioscopy correctly identified all 38 malignant strictures in this series.

Intraductal ultrasonography
Intraductal ultrasonography (IDUS) is a promising imaging modality for the evaluation of a variety of biliary disorders.[41,42] Intraductal ultrasonography does not provide definite diagnoses. However, the characterization of biliary strictures provided by IDUS can be used in combination with other diagnostic approaches to develop appropriate therapeutic strategies. Intraductal ultrasonography can provide the local staging to select patients with cholangiocarcinoma who benefit from surgical resection.[43–46] Recently, Stavropoulos et al.[47] reported that intraductal ultrasonography increased the
In these published series, the sensitivity of Fluorescence EUS-FNA may. This advanced techniques for providing a definitive diagnosis of neous transhepatic procedures are the most common Tissue collection during endoscopic and/or percutaneous transhepatic procedures are the most common. Therefore, negative cytological results do not from bile duct strictures is the poor quality of cytologic samples. Therefore, negative cytological results do not permit reliable exclusion of malignancy.

**Histology/cytology**

Clinical signs of bile duct obstruction

Transabdominal ultrasonography

CT/MRI

ERCP/PTC including: brush cytology forceps biopsy

Histology/cytology positive

Histology/cytology negative

Repeated tissue sampling using a cholangioscope

Histology/cytology positive

Histology/cytology negative

Repeated tissue sampling:

US guided puncture

CT guided puncture

EUS guided puncture

Planning therapeutic strategy

Figure 1 The diagnostic algorithm in patients with suspected extrahepatic bile duct obstruction.

Fluorescence in situ hybridization (FISH)

Recently, investigators have attempted to improve diagnostic assessment with an advanced cytological technique for the detection of malignant pancreaticobiliary strictures. Fluorescence in situ hybridization (FISH) has been shown to increase the sensitivity for the diagnosis of malignant pancreaticobiliary strictures compared to conventional cytology. Kipp et al used a multitarget FISH probe set which has previously shown high impact in monitoring recurrent urothelial carcinoma. This advanced technique identifies malignant cells by detecting aneusomy and deletion of the locus 9p21. By applying this technique for brush cytology and bile aspirate specimens in 131 patients with bile duct strictures (including 71 with primary sclerosing cholangitis, FISH analysis showed sensitivity of 35% and specificity of 91%. When patients with primary sclerosing cholangitis were excluded, sensitivity for malignancy detection by FISH was 16%. This indicates that probe sets specific for biliary neoplasms will be required for higher sensitivity. However, not all malignant tumors present aneusomy or aneuploidy. In the biliary tract, the percentage of cancers displaying aneuploidy has been estimated to be approximately 80%.

**CONCLUSION**

Figure 1 demonstrates the diagnostic algorithm used in our hospital for patients with suspected extrahepatic bile duct obstruction. Cholangiocarcinomas are still difficult to diagnose. In the future we need better early detection methods including molecular markers and improved histological techniques. Furthermore, new imaging and endoscopic techniques should be
developed to improve the diagnostic accuracy and tumor extension.

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