Phenotypic Diversity of Intrahepatic and Extrahepatic Cholangiocarcinoma on Aspiration Cytology and Core Needle Biopsy

Case Series and Review of the Literature

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BACKGROUND. Cholangiocarcinoma (CC) represents approximately 10% of primary liver malignancies and can mimic metastatic adenocarcinoma.

METHODS. The authors retrospectively reviewed the cytopathology files at the University of Texas Medical Branch to identify patients who were diagnosed with intrahepatic or extrahepatic CC by aspiration cytology between 1995 and 2004. Brush cytology specimens of extrahepatic CC were excluded. All diagnoses were confirmed as CC by clinical, imaging, and histopathologic findings and by chart review.

RESULTS. Cytopathology files from 13 patients with CC diagnosed by FNA were retrieved. The male:female ratio was 5:8, and the patients ranged in age from 29 years to 74 years (mean age, 59 years). In 12 of 13 patients, aspirates were obtained by ultrasound guidance; and, in 1 patient, computed tomography guidance was used. Three patients had aspirates only, 10 patients also had core biopsies, and 1 patient had cell block preparations. The phenotypic distribution of CC according to the World Health Organization (WHO) histologic classification was 9 adenocarcinoma (intrahepatic), not otherwise specified (NOS) (69%); 2 gastric foveolar type (extrahepatic) CCs (15%); 1 intestinal type (extrahepatic) CC (8%); and 1 sarcomatous/spindle cell type (intrahepatic) CC (8%). One adenocarcinoma, NOS was well differentiated CC with bland tubular architecture, and one was pleomorphic. Ancillary histochemical and immunochemical stains were performed on 5 of 13 specimens, which included 4 core biopsies and 1 aspirate with Mucicarmine positivity (3 specimens), carcinoembryonic antigen positivity (3 specimens), and a cytokeratin 7 (CK7)-positive/CK20-negative pattern (2 specimens). The 1 sarcomatous/spindle cell type CC was chromogranin-negative and low molecular weight keratin (cell adhesion molecule 5.2)-positive, which excluded metastatic carcinoid.

CONCLUSIONS. Classification of intrahepatic and extrahepatic CC in aspiration cytology specimens was achieved in a reliable manner concordant with the WHO histologic classification. Special types of CC with bland nuclear features posed a diagnostic challenge on cytologic evaluation, particularly the well differentiated CC with bland tubular architecture and the gastric foveolar type CC with mucin-producing tumor cells. The addition of core biopsy and/or ancillary studies, such as histochemical and immunochemical stains, were helpful in reaching the correct diagnosis.

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KEYWORDS: cholangiocarcinoma, intrahepatic, extrahepatic, World Health Organization, fine-needle aspiration, cytology.

The vast majority of malignant neoplasms that involve the liver are metastatic. Cholangiocarcinoma (CC) is a relatively uncommon primary malignant glandular tumor of the liver that is second in
frequency to hepatocellular carcinoma in adults. It affects approximately 3000 people annually in the U.S. and constitutes 15% of liver malignancies worldwide, with a greater prevalence in parts of Southeast Asia. CCs are more common in men in the fifth to seventh decades of life and can arise anywhere in the biliary tree. Intrahepatic CC arises in the intrahepatic bile ducts and also has been referred to as peripheral CC, intrahepatic bile duct carcinoma, and cholangiocellular carcinoma. Extrahepatic CC arises in the extrahepatic bile ducts, the periampullary region, and the gallbladder, with the majority of tumors originating in the upper one-third of the extrahepatic ductal system near the origin of the cystic duct. Several etiologic factors have been associated with CC, including choledochal cyst, clonorchiasis, hepatolithiasis, previous exposure to thorium dioxide, sclerosing cholangitis, and ulcerative colitis.

Fine-needle aspiration (FNA) biopsy performed under image guidance is a reliable method for the evaluation of deep-seated masses in the liver. Cytologically, CC is indistinguishable from adenocarcinomas arising elsewhere and, thus, needs to be differentiated from metastases to the liver. The addition of core biopsy is regarded as a helpful coadjuvant to aspiration cytology for the diagnosis of localized hepatic masses. Although the histologic appearances of the different types of intrahepatic and extrahepatic CC have been documented well in the literature, to our knowledge, there is no detailed description of the corresponding CC types on the basis of cytomorphology. The objective of the current study was to describe the cytologic appearances of the different CC types based on clinical, imaging, and cytohistologic observations derived from the fact that 10 of 13 specimens in our series included a core biopsy in addition to aspiration cytology. A review of the recent literature is included.

MATERIALS AND METHODS
A retrospective review of the cytopathology files was conducted at the University of Texas Medical Branch at Galveston for both intrahepatic and extrahepatic CCs that were diagnosed by aspiration cytology, with and without concomitant core biopsy, between 1995 and 2004. In total, files on 13 patients with CC diagnosed by FNA with (10 patients) and without (3 patients) core biopsy were retrieved for review. Patients who had CC diagnosed by brush cytology were excluded from this study. Metastatic adenocarcinoma or patients with symptoms suggestive of metastasis to the liver based on chart review also were excluded. All materials in this study were confirmed as intrahepatic or extrahepatic CC by clinical, imaging, and histopathologic findings.

RESULTS
In total, specimens from 13 patients with CC were retrieved from our cytopathology files with a male: female ratio of 5:8 and an age range of 29–74 years (mean age, 59 years). In 12 patients, aspirates were obtained by ultrasonographic (US) guidance of the needle; and, in 1 patient, computed tomography (CT) guidance was used. Ten patients underwent concomitant core biopsies in addition to aspirates; and, in 1 patient, cell block preparations also were performed. Based on the World Health Organization (WHO) classification for intrahepatic CC13 and tumors of the gallbladder and extrahepatic bile ducts,14 the phenotypic distribution of CCs in our cytology series was as follows: 9 tumors (69%) were classified as adenocarcinoma (intrahepatic), not otherwise specified (NOS); 2 tumors (15%) were classified as foveolar (extrahepatic); 1 tumor (8%) was classified as intestinal (extrahepatic); and 1 tumor (8%) was classified as the sarcomatous/spindle cell type (intrahepatic) (Table 1).

Cytologic Findings of CC Types
Adenocarcinoma, NOS (intrahepatic)
Cytologically, CC of the “adenocarcinoma, NOS” type is similar to adenocarcinomas that arise elsewhere in the pancreaticobiliary system. Aspirate smears demonstrated three-dimensional clusters of atypical ductal epithelial cells that had small-to-variable amounts of cytoplasm along with loss of nuclear polarity, irregular nuclear membranes, and prominent nucleoli. Tumor cells were arranged in acini focally (Fig. 1A,B) and often revealed evidence of mucin production. Occasionally, the malignant cells displayed significant nuclear pleomorphism and irregular nuclear contours (Fig. 2A).

Sarcomatous/spindle cell type (intrahepatic)
Aspirate smears of the sarcomatous/spindle cell variant of CC exhibited relatively small, ovoid-to-spindle, neoplastic cells with scant cytoplasm, hyperchromatic nuclei, high nuclear:cytoplasmic ratios, and minute nucleoli. Tumor cells often were arranged in small, loosely cohesive aggregates (Fig. 2B,C).

Foveolar type (extrahepatic)
Aspirate smears of foveolar type CCs demonstrated malignant cuboidal-to-columnar epithelial cells with relatively bland nuclear features, low nuclear:cytoplasmic ratios, and abundant, mucin-secreting cytoplasm that resembled gastric foveolar type epithelium (Fig. 3A,B).
**Intestinal type (extrahepatic)**

On aspirates, intestinal type CC resembled colonic adenocarcinoma. Smears exhibited cigar-shaped nuclei with marked atypia, crowding, and pseudostratification (Fig. 4A,B). The background often was necrotic.

**Histologic Findings**

**Adenocarcinoma, NOS (intrahepatic)**

A common variant of CC, adenocarcinoma, NOS reveals a heterogeneity of histologic features and degree of differentiation. Malignant cells range from cuboidal to columnar and can be pleomorphic. Increased nuclear:cytoplasmic ratios and prominent nucleoli often are observed. Tumors display tubular, cord-like, and micropapillary arrangements. The neoplastic cells have pale, eosinophilic, or vacuolated-to-clear cytoplasm (Fig. 1C) and can resemble goblet cells. The malignant glands often are surrounded by abundant fibrous stroma, which is a distinctive feature of CC. Fibrosis is characterized by activated perisinusoidal...
cells, which are activated myofibroblasts. These cells incorporate into the tumor and produce extracellular matrix proteins that lead to fibrosis. Secretion of mucus by neoplastic glands can be proven in the majority of tumors by using appropriate histochemical stains for mucin. Poorly differentiated CCs of the adenocarcinoma type can display pleomorphic features, and their differentiation from other malignancies that affect the liver, including metastases, can be challenging.

**Sarcomatous/spindle cell type (intrahepatic)**
The spindle cell variant of CC resembles spindle cell sarcomas like fibrosarcoma and malignant fibrous histiocytoma and, focally, may exhibit carcinomatous foci. Because of small cell size and frequent ovoid-to-spindle nuclei, metastatic carcinoid also has to be considered in the differential diagnosis of this CC type. “Salt-and-pepper” type chromatin and reactivity with neuroendocrine immunostains are features of carcinoid tumors that usually are not observed in CC.

**Foveolar type (extrahepatic)**
In the foveolar variant of CC, the glands are lined by a single layer of tall columnar cells with abundant mucin-containing cytoplasm, basal nuclei with relatively bland nuclear features, small nucleoli, low mitotic rate, and focal polypoid-to-lobular architecture (Fig. 3C).
Intestinal type (extrahepatic)
The intestinal variant of CC resembles colonic adenocarcinoma. It is composed of malignant tubular glands or papillary structures lined predominantly by tumor cells with an intestinal phenotype, with or without the presence of goblet cells, along with ovoid or cigar-shaped, hyperchromatic nuclei in a pseudostratified pattern (Fig. 4C).

Histochemical and Immunohistochemical Findings
Ancillary histochemical and immunohistochemical stains were performed on 5 of 13 specimens, including 4 core biopsies and 1 specimen that consisted of aspirates only. Three CCs (two adenocarcinoma, NOS and one intestinal type) were positive for Mucicarmine (Fig. 5A) and coexpressed carcinoembryonic antigen (CEA) immunostain. A cytokeratin (CK) immunostain profile of CK7-positive (Fig. 5B) and CK20-negative was identified in 2 tumors (both adenocarcinoma, NOS). The 1 spindle cell type CC was chromogranin-negative (Fig. 5C) and expressed low-molecular-weight (cell adhesion molecule 5.2) keratin, which helped to exclude the possibility of metastatic carcinoid (Table 1).

DISCUSSION
CC is a relatively rare form of adenocarcinoma that arises on biliary epithelium at intrahepatic or extrahepatic locations. Worldwide, CC represents up to 15% of primary liver malignancies, and it has a higher prevalence in Asia. Risk factors associated with CC include cystic dilatation of the bile ducts and choledochal cyst; hepatolithiasis; infestation with the intrahepatic duct fluke *Clonorchis sinensis*, which occurs commonly in Southeast Asia; ulcerative colitis; primary sclerosing cholangitis; and previous exposure to the contrast medium thorium dioxide. Although the latter is associated strongly with the development of CC, the other risk factors account for only a minority of CC.

CC usually affects older individuals; however, it can arise in younger patients, particularly if it is associated with known risk factors. In > 90% of patients with CC, jaundice is the usual clinical presentation; other findings include weight loss, abdominal discomfort, elevated levels of serum bilirubin and alkaline phosphatase, and occasionally elevated serum tumor markers, such as CEA and CA19-9.

Imaging studies, such as CT and US, can suggest CC and its anatomic origin; however, with diffusely infiltrating tumors, it may be impossible to ascertain the site of origin. With the addition of contrast enhancement, CT scans of intrahepatic CC usually reveal a characteristic hypodense hepatic mass with peripheral enhancement and biliary dilatation. US also has proven to be highly sensitive in the detection of intrahepatic CC and often reveals a hepatic tumor mass.
with dilated intrahepatic bile ducts and normal common bile duct. Endoscopic US and endoscopic retrograde cholangiopancreatography generally are regarded as very sensitive for the detection of malignant obstruction in extrahepatic CC, whereas CT scans and magnetic resonance images usually reveal characteristic appearances that suggest intrahepatic neoplasms. A conclusive diagnosis of hepatic neoplasm cannot be made without microscopic examination of the lesion. Pathologic confirmation is required not only for diagnosis but also for the exclusion of hepatocellular carcinoma and metastatic disease to the liver. FNA, with or without core biopsy, is an effective method for establishing a confident morphologic diagnosis of lesions arising in or involving the liver with minimal morbidity. The aspiration cytology findings of CC, both in primary tumors of the liver and in metastases to other anatomic locations, have been described sparsely in the literature. To our knowledge, a detailed description of the cytologic appearance of the different histologic types of CC has not been published previously.

The aspiration cytology of the adenocarcinoma, NOS type of CC varies from well differentiated to poorly differentiated and shares similar morphologic features with other adenocarcinomas, including metastasis to the liver, as described above. Histochecmical evidence of mucin production can be seen in most CCs by using mucicarmine (Fig. 5A), periodic acid–Schiff with diastase, and Alcian blue stains. In addition, CC exhibits a cytokeratin pattern of CK7-positive and CK20-negative (Fig. 5B) immunostaining that helps to differentiate these tumors from malignancies of colonic and lower gastrointestinal origins. Greater than two-thirds of the CCs in the current series were classified as the adenocarcinoma, NOS type, with 1 tumor (Patient 4) that exhibited markedly irregular nuclear contours and poorly differentiated features consistent with the pleomorphic subtype of CC (Fig. 2A). Another tumor that was classified as an adenocarcinoma was an example of intrahepatic, well differentiated CC that was characterized by the presence of small neoplastic glands with bland nuclear features both cytologically and on tissue sections (Fig. 6A,B). Because of their usual bland morphology, intrahepatic, well differentiated CC can be extremely difficult to differentiate from bile duct adenomas on the basis of pure cytology. Correlation with imaging findings is crucial, because adenomas of the bile duct are located superficially in the liver and usually measure < 1 cm. Conversely, larger hepatic lesions with the presence of necrosis are indicative of malignancy, such as intrahepatic, well differentiated CC. To complicate matters, patients who have metastatic, well differentiated adenocarcinoma of the liver from the breast and other anatomic origins can present with “bland” cytomorphology almost identical to that of some intrahepatic CCs. Moreover, the pattern of CK7 reactivity with CK20 nonreactivity usually seen in CCs also can be expressed by adenocarcinomas arising in other anatomical locations, such as breast, lung, pancreas, and gallbladder. In such patients, correlation with clinical and imaging findings, as well as comparison with morphology of any previously diagnosed malignancy, is essential to establish an accurate diagnosis.

On aspiration cytology the sarcomatous/spindle cell variant of intrahepatic CC can be difficult to differentiate from other neoplasms that have spindle cell morphology, such as sarcomas and metastatic neuroendocrine tumors. Histologically, spindle cell CCs often exhibit foci with carcinomatous differentiation, including squamous carcinoma. Immunoreactivity with cytokeratins and a pattern of CK7 positivity with CK20 nonreactivity are characteristic of CC. Different from spindle cell CCs, sarcomas usually are reactive with vimentin; and carcinoids express neuroendocrine markers, such as chromogranin, synaptophysin, and neuron-specific enolase.

Because of their usual bland morphologic features that resemble gastric epithelium, CCs of the extrahe-

FIGURE 6. Fine-needle aspiration (FNA) and core biopsy specimens from a cholangiocarcinoma that was classified as an intrahepatic “adenocarcinoma, not otherwise specified” show well differentiated, neoplastic acini with bland nuclear features (A) (Papanicolaou stain) in a fibrotic stroma (B) (H & E stain). (C) An FNA liver specimen shows metastatic tubular carcinoma from the breast (Romanowski stain). Original magnification ×400 (A, B); ×200 (C).
patic foveolar type have been confused with adenomas of the gallbladder.\textsuperscript{16} However, imaging findings usually afford the distinction of extrahepatic duct from gallbladder lesions. In addition, the occurrence of focal areas that exhibit less tumor differentiation and the presence of perineural invasion on deeper sections helps to distinguish between these two entities. Similar to what occurs with normal gastric foveolar epithelium, the mucin-containing cells of the foveolar type of CC express CK8, CK20, and cathepsin D immunostains. Furthermore, the presence of mucin can be highlighted with stains like mucicarmine (Fig. 5A), periodic acid–Schiff with diastase, and Alcian blue.\textsuperscript{16} On aspiration cytology, the cells of foveolar type CC are identical to those seen in core biopsy (Fig. 3A–C).

The intestinal type of CC has to be distinguished from metastatic colonic carcinoma and other mucus-secreting neoplasms, especially colloid and signet ring cell adenocarcinoma, that arise elsewhere in the gastrointestinal tract. Colloid carcinoma exhibits large pools of mucin with floating islands of malignant cells, whereas signet ring cells are the hallmark of signet ring cell adenocarcinoma. Although mucin is abundant in both of these malignant subtypes, classic goblet cells either are absent or are found rarely. In addition, the characteristic intestinal gland formation with pseudostratification and cigar-shaped nuclei (Fig. 4A–C) is not a prominent microscopic feature of colloid or signet ring adenocarcinomas.\textsuperscript{14} New improvements in imaging methods permit safer and more accurate pathologic diagnoses of deep-seated lesions by means of image-guided (CT, US, or endoscopic US) FNA with or without core biopsy. Despite its known limitations in the diagnosis of nonneoplastic and benign, diffuse hepatocytic lesions, FNA cytology generally is advocated as the initial approach for focal lesions of the liver.\textsuperscript{38} However, a review of the medical literature revealed varying degrees of support with regard to the effectiveness of aspirates versus needle-core biopsy, with some authors suggesting that core biopsy should be the only method used\textsuperscript{39} and others advocating the use of FNA for sampling liver masses and other abdominal masses.\textsuperscript{38–41} FNA by an on-site cytopathologist for the assessment of adequacy and preliminary evaluation of aspirates has been associated with improvements in overall accuracy.\textsuperscript{42–45} The current series consisted of 13 patients who had CC diagnoses established by FNA cytology only (3 of 13 patients) or by FNA cytology and core biopsy (10 of 13 patients) (Table 1). Aspirates were adequate for diagnosis of CC in 12 of 13 patients (92%) overall and in 9 of 10 patients (90%) who had concomitant core biopsies. On core biopsy, the 1 patient who had suboptimal diagnostic material from aspirates (Patient 9) proved to have a CC with marked desmoplastic reaction, which explains its low cellular yield in FNA material. Conversely, all 10 patients in our series who underwent core biopsies had histologic features of CC revealed in biopsy tissue sections. In a recent study by Stewart et al., the sampling of liver lesions revealed 89% and 100% sensitivity for core biopsy and FNA alone, respectively, with a combined sensitivity of 100%.\textsuperscript{46}

The final choice in the diagnostic procedure for a given deep-seated lesion ultimately should be determined by a combination of factors, such as anatomic location of the tumor mass, personal preferences of the radiologist, prevailing experience at the institution, and the clinical differential diagnosis.\textsuperscript{38} Although we are sensitive to the fact that not all institutions can afford to perform image-guided FNA of deep-seated lesions with an on-site pathologist, at our institution, the preferred approach is initial image-guided FNA with on-site cytopathologic evaluation and triaging of the patient. The addition of core biopsy—as a complement to aspirates—is chosen for certain patients when it may help the diagnosis. Such an approach led to 100% combined FNA/core biopsy sensitivity in our series of patients. In addition, the use of core biopsy as a coadjuvant to the FNA was particularly helpful in subtyping the different CC variants.

This study further emphasized the value of FNA cytology as a reliable method for the diagnosis of neoplastic lesions arising in or involving the liver. The use of core biopsy as a coadjuvant to aspiration cytology can be helpful in the diagnosis and subtyping of CC. Based on the results from correlating aspirates with concomitant core biopsy materials, this retrospective study suggests that the cyologic identification of different histologic types of CC, as described by the WHO classification,\textsuperscript{13,14} is possible, and such cytomorphologic findings are illustrated in the images provided. However, some histologic types of CC can pose significant diagnostic challenges on cyologic evaluation, particularly the well differentiated, intrahepatic CC with tubular gland architecture and the extrahepatic, foveolar-type CCs with bland, mucin-producing tumor cells that resemble benign gastric epithelium. In such instances, the value of correlating clinical and imaging findings cannot be overemphasized. The addition of core biopsy and ancillary histochemical and immunohistochemical stains may be required for such difficult cases.
REFERENCES

1. Parkin DM, Ohshima H, Srivatanakul P, Vatanasapt V. Cholangiocarcinoma: epidemiology, mechanisms of carcinogenesis, and prevention. Cancer Epidemiol Biomarkers Prev. 1993;2:537–544.

2. Yiannakis PH, Patrikeos A, Ford HT, Davis CL. Metastatic cholangiocarcinoma with an occult primary. Clin Oncol (R Coll Radiol). 1995;7:394–.

3. Thuluvath PJ, Rai R, Venbrux AC, Yeo CJ. Cholangiocarcinoma: a review. Gastroenterologist. 1997;5:306–315.

4. Ishak KG, Goodman ZD, Stocker JT. Intrahepatic cholangiocarcinoma and other malignant biliary tumors. In: Ishak KG, Goodman ZD, Stocker JT, editors. Fascicle 31. Atlas of tumor pathology: tumors of the liver and intrahepatic bile ducts. Washington, DC: American Registry of Pathology, Armed Forces Institute of Pathology, 2001:245–270.

5. Tompkins RK, Thomas D, Wile A, Longmire WP. Prognostic factors in bile duct carcinoma: an analysis of 96 cases. Ann Surg. 1981;194:474–455.

6. Albores-Saavedra J, Henson DE, Klimstra DS. Malignant epithelial tumors of the extrahepatic bile ducts. In: Albores-Saavedra J, Henson DE, Klimstra DS, editors. Fascicle 27. Atlas of tumor pathology: tumors of the gallbladder, extrahepatic bile ducts, and ampulla of Vater. Washington, DC: American Registry of Pathology, Armed Forces Institute of Pathology, 2000:181–190.

7. Lipsett PA, Pitt HA, Colombani PM, Boitnott JK, Cameron JL. Choledochal cyst disease. A changing pattern of presentation. Ann Surg. 1994;220:644–652.

8. Lee JH, Yang HM, Bak UB, Rim JH. Promoting role of Clonorchis sinensis infection on induction of cholangiocarcinoma during two-step carcinogenesis. Korean J Parasitol. 1994;32:13–18.

9. Rubel LR, Ishak KG. Thorotras-t-associated cholangiocarcinoma: an epidemiologic and clinicopathologic study. Cancer. 1982;50:1408–1415.

10. Knecnhle SJ, D’Alessandro AM, Harms BA, Pirsch JD, Belzer FO, Kalayoglu M. Relationships between sclerosing cholangitis, inflammatory bowel disease, and cancer in patients undergoing liver transplantation. Surgery. 1995;118:615–619.

11. Pitt HA, Dooley WG, Yeo CJ, Cameron JL. Malignancies of the biliary tree. Curr Probl Surg. 1995;32:1–90.

12. Suen KC. Diagnosis of primary hepatic neoplasms by fine-needle aspiration cytology. Diagn Cytopathol. 1982;2:99–109.

13. Nakamura Y, Sripa B, Vatanasapat V, Leon A.S-T, Ponchon T, Ishak KG. Intrahepatic cholangiocarcinoma. In: Hamilton SR, Aaltonen LA, editors. Pathology and genetics. Tumours of the digestive system. World Health Organization classification of tumours. Lyon: IARC Press, 2000:173–180.

14. Albores-Saavedra J, Scaccez JC, Wittekind C, et al. Carcinoma of the gallbladder and extrahepatic bile ducts. In: Hamilton SR, Aaltonen LA, editors. Pathology and genetics. Tumours of the digestive system. World Health Organization classification of tumours. Lyon: IARC Press, 2000:206–214.

15. Terada T, Makimoto K, Terayama N, Suzuki Y, Nakamura Y. Alpha-smooth muscle actin-positive stromal cells in cholangiocarcinomas, hepatocellular carcinomas and metastatic liver carcinomas. J Hepatol. 1996;24:706–712.

16. Albores-Saavedra J, Delgado R, Henson DE. Well-differentiated adenocarcinoma, gastric foveolar type, of the extrahepatic bile ducts: a previously unrecognized and distinctive morphologic variant of bile duct carcinoma. Ann Diagn Pathol. 1999;3:75–80.

17. Soper P, Blauemke DA, Reichle R, et al. Imaging of intrahepatic cholangiocarcinoma: 2. Hilar cholangiocarcinoma. AJR Am J Roentgenol. 1995;165:1433–1436.

18. Albores-Saavedra J, Henson DE, Klimstra DS. Dysplasia, carcinoma in situ, and invasive carcinoma of the extrahepatic bile ducts. In: Fascicle 27. Atlas of tumor pathology: tumors of the gallbladder, extrahepatic bile ducts, and ampulla of Vater. Washington, DC: American Registry of Pathology, Armed Forces Institute of Pathology, 2000:191–215.

19. Valls C, Guma A, Puig I, Sanchez A, Andia E, Serrano T, Figueras J. Intrahepatic peripheral cholangiocarcinoma: CT evaluation. Abdom Imaging. 2000;25:490–496.

20. Karstrup S. Ultrasound diagnosis of cholangiocarcinoma at the confluence of the hepatic ducts (Klatskin tumours). Br J Radiol. 1988;61:987–990.

21. Dancygier H, Natterman C. The role of endoscopic ultrasound in biliary tract disease: obstructive jaundice. Endoscopy. 1994;26:800–802.

22. Ros PR, Buck JL, Goodman ZD, Ros AM, Olmsted WW. Intrahepatic cholangiocarcinoma: radiologic-pathologic correlation. Radiology. 1988;167:689–693.

23. Fan YZ, Yamashita Y, Harada M, et al. Intrahepatic cholangiocarcinoma: spin-echo and contrast-enhanced dynamic MRI imaging. AJR Am J Roentgenol. 1993;161:313–317.

24. Dalton-Clarke HJ, Pearse E, Krause T, McPherson GAD, Benjamin IS, Blumgart LH. Fine needle aspiration cytology and Exfoliative biliary cytology in the diagnosis of hiliar cholangiocarcinoma. Eur J Ultrasound. 1996;12:143–145.

25. Fritscher-Ravens A, Broering DC, Knoefel WT, et al. EUS-guided fine-needle aspiration of suspected hilar cholangiocarcinoma in potentially operable patients with negative brush cytology. Ann J Gastroenterol. 2004;99:45–51.

26. Chawla S, Malik N, Wig JD, Kochhar R, Gupta SK, Suri S. Cholangiographically guided aspiration cytology in the management of malignant biliary obstruction. Indian J Gastroenterol. 1989;8:95–96.

27. Desa LA, Akosa AB, Lazzara S, Domizio P, Krausz T, Benjamin IS. Cytodiagnosis in the management of extrahepatic biliary stricture. Gut. 1991;32:1188–1191.

28. Fritscher-Ravens A, Broering DC, Sriman PVJ, et al. EUS-guided fine-needle aspiration cytodiagnosis of hilar cholangiocarcinoma: a case series. Gastrointest Endosc. 2000;52:534–540.

29. Sampatanukul P, Leong AS-Y, Kosolbhand P, Tangkijvanich P. Proliferating ductules are a diagnostic discriminator for intrahepatic cholangiocarcinoma in FNA biopsies. Diagn Cytopathol. 2000;22:359–365.

30. Tot T. Cytokeratins 20 and 7 as biomarkers: usefulness in the confluence of the hepatic ducts (Klatskin tumours). Ear Nose Throat J. 2002;81:776–778.

31. Tot T. Cytokeratins 20 and 7 as biomarkers: usefulness in the confluence of the hepatic ducts (Klatskin tumours). Ear Nose Throat J. 2002;81:776–778.
34. Cho C, Rullis I, Rogers LS. Bile duct adenomas as liver nodules. *Arch Surg.* 1978;113:272–274.
35. Jang KY, Kang MJ, Lee DG, Chung MJ. Utility of thyroid transcription factor-1 and cytokeratin 7 and 20 immunostaining in the identification of origin in malignant effusions. *Anal Quant Cytol Histol.* 2001;23:400–404.
36. Jain D. Diagnosis of hepatocellular carcinoma. Fine needle aspiration cytology or needle core biopsy. *J Clin Gastroenterol.* 2002;35(Suppl 2):S101–S108.
37. Nyman RS, Cappelen-Smith J, Brismar J, von Sinner W, Kagevi I. Yield and complications in ultrasound guided biopsy of abdominal lesions. Comparison of fine needle aspiration biopsy and 1.2 mm needle core biopsy using an automated gun. *Acta Radiol.* 1995;36:485–490.
38. Pitman MB. Fine needle aspiration biopsy of the liver. Principal diagnostic challenges. *Clin Lab Med.* 1998;18:483–506.
39. Fornari F, Civardi G, Cavanna L, et al. Ultrasonically guided fine-needle aspiration biopsy: a highly diagnostic procedure for hepatic tumors. *Am J Gastroenterol.* 1990;85:1009–1013.
40. Jacobsen GK, Gammelgaard J, Fugl M. Coarse needle biopsy versus fine needle aspiration biopsy in the diagnosis of focal lesions of the liver. *Acta Cytol.* 1983;27:152–156.
41. Livraghi T, Sangalli G, Giordano F, Vettori C. Fine needle aspiration versus fine cutting needle, and comparison between smear cytology, inclusion cytology and microhistology in abdominal lesions. *Tumori.* 1988;74:361–364.
42. Klapman JB, Logroño R, Dye CE, Waxman I. Clinical impact of on-site cytopathology interpretation on endoscopic ultrasound-guided fine needle aspiration (EUS-guided FNA). *Am J Gastroenterol.* 2003;98:1289–1294.
43. Logroño R, Waxman I. Interactive role of the cytopathologist in EUS-guided fine needle aspiration: an efficient approach. *Gastrointest Endosc.* 2001;54:485–490.
44. Saleh HA, Khatib G. Positive economic and diagnostic accuracy impacts of on-site evaluation of fine needle aspiration biopsies by pathologists. *Acta Cytol.* 1996;40:1227–1230.
45. Jhala NC, Jhala D, Eltoum I, et al. Endoscopic ultrasound-guided fine-needle aspiration biopsy: a powerful tool to obtain samples from small lesions. *Cancer (Cancer Cytopathol).* 2004;102:239–246.
46. Stewart CJR, Coldewey J, Stewart IS. Comparison of fine needle aspiration cytology and needle core biopsy in the diagnosis of radiologically detected abdominal lesions. *J Clin Pathol.* 2002;55:93–97.