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Bile Duct Involvement by Hepatocellular Carcinoma: A Rare Occurrence and Poor Prognostic Indicator in Bile Duct Brushing Samples

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Abstract

Background: Hepatocellular carcinoma (HCC) rarely involves the biliary tree and may be inadvertently sampled on bile duct brushings (BDBs).

Design: 5 institutions' pathology archives were searched for BDBs with HCC involvement.

Results: 17 BDBs from 14 patients had a M:F ratio of 6:1, median age 59.5 years (range 22-80), median hepatic tumor size 6.2cm (range 2.2-13.0cm). HCC risk factors included viral hepatitis (n=5), cirrhosis (n=5), hemochromatosis (n=1) and alcoholic steatohepatitis (n=1). Jaundice with elevated bilirubin, liver enzymes, and alpha-fetoprotein were common. ERCP showed bile duct dilatation, polypoid intraductal masses (n=5), clots/debris (n=2) or strictures (n=4). All BDBs had single and clustered large cells with naked atypical nuclei, granular cytoplasm, high nuclear-to-cytoplasmic ratios, and nuclei with prominent macronucleoli. Less common findings included clear/microvesicular cytoplasm (35%), papillae (29%) and anisonucleosis (35%). Classical HCC features (widened trabeculae (35%), endothelial wrapping (24%), multinucleation (24%) and cytoplasmic bile pigment (35%)) were uncommon. 11 BDBs were diagnosed as malignant (10 HCC ,1 cholangiocarcinoma), two as atypical and one as negative; 2/3 had polysomy on F.I.S.H. and 71% died of disease at median 3.5 months. **Conclusion:** HCC may extend into intra/extrahepatic biliary tree, causing masses/strictures that may be sampled on BDB. Although cytologically malignant, classical features of HCC are uncommon, which can cause misdiagnosis. Cytopathologists should be mindful of this differential when evaluating BDBs, particularly when concomitant liver masses and/or HCC risk factors are present. Because of the associated high mortality and rapid demise its' presence should be clearly conveyed in pathology reports.

Key words: hepatocellular; carcinoma; bile duct; brushing;

Concise 2 sentence summary: Biliary tree involvement by hepatocellular carcinoma (HCC) may result in tumor sampling by bile duct brushing. Cytopathologists should be mindful of this differential, particularly when concomitant liver masses and/or HCC risk factors are present.

Background

The diagnosis of neoplasms in the biliary tree remains a clinical and pathologic challenge, with brush cytology and (less frequently) small biopsies being the most frequently used,

albeit imperfect, diagnostic tests. Cytologic diagnosis of malignancy is often confounded by well-differentiated cytologically bland carcinoma, instrumentation- and cholangitis-related reactive changes.[1,2]

Nonetheless, most biliary tract tumors that are diagnosed on bile duct brushings (BDBs) or intraductal biopsy are either pancreatic (pancreatic ductal adenocarcinoma) or biliary (intra- and extrahepatic cholangiocarcinoma) in origin. [3-6] Other sources of positive results are intraductal papillary neoplasms of bile duct (IPNB), primary tumoral intraductal neoplasms of the intra- and extrahepatic biliary tree and metastatic non-pancreatobiliary tumors from the upper and lower gastrointestinal tract, and even lung.[4-6] In addition to metastases from distant sites, the biliary tract may also be involved by direct extension of hepatocellular carcinoma (HCC).

Intraductal spread of HCC in the biliary tract is an unusual source of malignant cells on BDB.[7,8,9-11] Whether this is due to the rarity of involvement or to cytopathologists' failure to recognize tumor in these specimens is unclear, but when contrasted with the number of new cases of HCC world-wide (estimated 841, 080 in 2018), it is surprising that this phenomenon is not more frequently encountered.[12] We sought to examine the clinicopathologic characteristics of patients with bile duct brushing samples demonstrating HCC.

Materials and Methods

A multi-institutional search of the pathology department archives of 5 large tertiary institutions (Beth Israel Deaconess Medical Center, Boston MA, Emory University, Atlanta, GA (IRB00095765), Mayo Clinic, Rochester MN, Memorial Sloan Kettering Cancer Center, NY, NY and University of Michigan, Ann Arbor MI) was conducted for bile duct brushings in which HCC was identified either on initial review or on re-review triggered by a concurrent or subsequent positive liver biopsy or resection. Clinicopathologic data, radiologic findings, cytogenetic and cytohistologic features from cytology samples and concurrent or

subsequent biopsies and/or resections (where available) was collected on all patients. Cytology specimens were re-examined for cytologic features that historically have been described in HCC including specimen hypercellularity, increased nuclear-to-cytoplasmic ratio, singly dispersed atypical naked nuclei, macronucleoli, multinucleated tumor cells, endothelial wrapping (well-defined vessels traversing tissue fragments), widened trabeculae > 2 cell plates in thickness and cytoplasmic bile pigment.[13,14] Additionally, the presence of malignant-appearing single cells with preserved granular "oncocytoid" or clear/microvesicular cytoplasm, nuclear pleomorphism or anisonucleosis, increased nuclear-to-cytoplasmic ratio, papillae, necrosis, multinucleated tumor giant cells and a 2-cell population of oncocytoid cells with abundant granular cytoplasm and ductal cells (singly dispersed columnar cells or honeycomb sheets) was noted.

Results

Clinical Characteristics

Of more than 5,000 bile duct brushings collected from 5 institutions over more than 20 years, 17 bile duct brushings with HCC were identified in 14 patients. Specimen contribution by institution was University of Michigan and Mayo Clinic, 2, Memorial Sloan Kettering Cancer Center, 3, Beth Israel Deaconess Medical Center, 4 and Emory University, 6. These 17sampleswere identified in 12 males and 2 females (M:F 6:1) of median age 59.5 years (range 22-80 years). Eight patients presented with jaundice, 13 had elevated serum bilirubin, all had elevated liver enzymes, five had elevated serum alpha fetoprotein levels and two had elevated serum CA19-9 and CEA. Risk factors for HCC were noted in 10 patients, including hepatitis B (n=2) and C (n=3) virus infection, hereditary hemochromatosis (n=1), alcoholic steatohepatitis (ASH) with cirrhosis (n=1), non-alcoholic steatohepatitis (NASH) (n=1) and cirrhosis without obvious etiology (n=2). Three patients were clinically suspected of having recurrent HCC at the time of BDB, because they had previously been diagnosed and successfully treated for HCC years earlier. These included one patient each with hemochromatosis, ASH and a 22-year old with a history of fibrolamellar HCC.

Radiologic Findings

Imaging results (including computerized tomography and magnetic resonance imaging) were available in all cases and are summarized in Table 1. A hepatic mass (or masses) was seen on imaging in 12 patients. Hepatic tumors ranged in size from 2.2 – 13.0 cm with a median size of 6.2 cm. Intrahepatic and/or extrahepatic bile duct dilatation +/- involvement of the common bile duct was seen in eight (57%) and portal vein thrombosis was seen in three (21%). Documented radiologic diagnoses included HCC (n=6), cholangiocarcinoma (n=6), and HCC vs ICC (n=2). One patient (case 5) had no hepatic or bile duct masses on imaging.

Endoscopic Retrograde Cholangiopancreatography (ERCP) Findings

ERCP results were available for review in 13 patients. These showed variable findings, including papillary or polypoid intraductal lesions (n=7) [described as papillary, polypoid, frond-like masses (n=5), debris (n=1) or clots (n=1)], as well as strictures of the hepatic or common bile duct without masses (n=4). Two patients had no intraductal masses, strictures or other duct abnormality on ERCP.

Cytologic Findings

All patients had bile duct brushings of the intrahepatic, extrahepatic or common bile duct, and two had brushings of both intrahepatic ducts and common bile duct. Sixteen samples had ThinPrep® slides (14 with cell blocks and 2 without) and one had hematoxylin and eosin-stained smears only. Variable immunohistochemical stains (including pancytokeratin, cytokeratin 7, arginase, hep-Par, and glypican 3) and reticulin were performed on 10 specimens. Eleven specimens were called malignant on cytology, one was misinterpreted as benign and 2 were called "atypical cells present." Of the 11 cases called malignant on brushing, 9 (82%) were diagnosed as HCC, 1 (9%) as "favor HCC" and 1 (9%) as adenocarcinoma (ICC).

Cytologic findings that were present in 80% - 100% of samples included 3-dimensional clusters and singly dispersed atypical cells with naked nuclei or abundant granular cytoplasm (Figure 1 – 2). Cells often had a high nuclear-to-cytoplasmic ratio (77%) with round to oval nuclei (nuclear irregularity was seen in 35%) and prominent central nucleoli in the majority (94%) of cases (Figure 1 – 2). Less common cytologic findings included necrosis (47%), a second population of benign-appearing ductal cells (47%), hypercellularity (41%), 4-fold or greater anisonucleosis (35%) (Figure 1 - 2),clear or bubbly microvesicular pale cytoplasm (35%) and papillary groups (29%) (Figure 3 - 4). Classical cytologic features of HCC such as widened trabeculae (35%), endothelial wrapping (24%), cytoplasmic bile pigment (24%), and multinucleated malignant cells (24%) were less frequently seen (Figure 2 – 3). Widened trabeculae were best seen on cell block, highlighted by reticulin stain and positive for hepatocellular differentiation markers arginase or hep-Par (Figure 4). Occasional cases (29%) showed variably sized "papillary groups" lined by epithelial cells with abundant eosinophilic granular to clear cytoplasm (Figure 4), focally resembling a steatohepatitic HCC (Figure 4). Cytologic features of the cases are highlighted in Figures 1 – 4.

Histologic Findings

Eleven patients had concurrent liver biopsies at the time of BDB. The diagnosis of HCC was confirmed in 10, including the fibrolamellar variant, and the tumors ranged from well to poorly differentiated. One tumor on biopsy showed a mixed HCC-cholangiocarcinoma but the corresponding bile duct brushings showed only large cells with granular cytoplasm and central round nuclei with macronucleoli consistent with the HCC component. Only one tumor was resected and showed a pT3b poorly differentiated HCC with lymph-vascular invasion and intraductal growth of tumor (Figure 4). The mixed HCC-cholangiocarcinoma case showed single intact cells and naked atypical nuclei as well as 3-dimensional clusters of malignant cells with abundant granular or clear cytoplasm and round to oval nuclei with macronucleoli (Figure 1) and focal nuclear membrane irregularity.

Fluorescence in-situ hybridization (F.I.S.H.)

F.IS.H. was performed on 3 specimens and was "positive" in 2 cases which both showed polysomy (1 by Vysis® UroVysion™ F.I.S.H. showed greater than 5 cells with 2 or more chromosomes [3, 7 and 17] and another by a Pancreatobiliary F.I.S.H. probe kit which showed gains of 2 or > loci of 1q21, 7p12, 8q24, and 9p21). Both F.I.S.H.-positive cases were poorly differentiated, one was 8.0cm and the other 11.3cm, and both patients had chronic viral hepatitis.

Follow-Up Information

Follow-up information was available for all patients and ranged from 0.2 months - 49 months, median 3.5 months. Ten (71%) patients died of disease, three (21%) were alive with disease at last follow-up and one (7%) was lost to follow-up at 49 months. Interestingly, of the 10 that died the median survival was 3.5 months (range 1 – 46 months) and six (60%) patients died 1 - 5 months after diagnosis. Of the six patients that died within 5 months of diagnosis one had a very small (2.0 cm) and purely intraductal HCC.

Discussion

The extension of hepatocellular carcinoma into the intra- and extra-hepatic biliary tree was first described in 1947 by Mallory et al[15] and is known historically by names such as "icteric-type hepatoma"[16] and "cholestatic HCC".[17] When this occurs, it leads to presentation with jaundice, elevated serum bilirubin and elevated liver enzymes, which was seen in more than half of our cohort. Serum alpha fetoprotein may or may not be elevated, which, in the absence of a liver mass, may delay diagnosis and confound radiologists and gastroenterologists. Obstructive jaundice as the main presentation in HCC occurs in 1-12% of patients.[18] Early identification is important as patients may benefit from early surgical intervention.[19]

Duct obstruction in HCC is either due to intraductal accumulation of clots (hemobilia) or debris, or intraductal tumor growth, duct wall invasion, or external compression by an expansile tumor.[19] The intraductal component may be contiguous with the hepatic tumor, or

is completely separate, with or without mucosal attachment.[20] There are even reports of HCC occurring entirely within the biliary tree without a discernable hepatic component, and is independent of size and differentiation.[21-23] Several of our cases were morphologically well-differentiated, almost half were 5.0cm or smaller and 14% showed pure intraductal growth. Most intraductal tumors are pure HCCs but mixed HCC-cholangiocarcinomas are also described.[24,25] Ductal involvement by HCC is not easily identifiable on CT and MRI, but is more obvious on ERCP which also allows direct visualization and sampling of polypoid masses or strictures.[19] Because of its rarity and subtle appearance tumors are either missed by gastroenterologists, or misinterpreted as cholangiocarcinoma or choledocholithiasis.[26,27] Intraductal involvement by HCC is associated with a poor prognosis,[19] which was seen in our cohort where 70% died within 6 months of diagnosis.

The presence of HCC in BDBs has also been described in isolated reports, with our study representing the largest series in the cytology literature. [9-11,25,28] The most frequent cytologic features that we identified were singly dispersed intact and 3-dimensional clusters of polygonal tumor cells with well-defined cell borders, relatively high nuclear-to-cytoplasmic ratio, abundant granular oncocytoid cytoplasm and round to oval central nuclei with variable chromatin (hyper- and hypochromatic) and prominent, often macro-, nucleoli. Widened trabeculae (which we defined as hepatic plates that were greater than 2 cells thick) with clearly delineated endothelial lining cells were only rarely seen, so too was intracytoplasmic bile, features that would typically favor HCC over cholangiocarcinoma. The fact that most of our samples were liquid-based preparations and had limited cellularity as well as necrosis may have contributed to difficulty in finding intact trabeculae and clinging endothelial cells. These features were best seen on cell block but the cell blocks were often paucicellular and bloody. Additionally, because of their monotony, more well-differentiated examples of HCC may be mistakenly classified as benign or indeterminate on brushings, which happened in three of our cases. Although most of our cases were accurately diagnosed on cytology, 60% required ancillary immunocytochemical stains including arginase

and hep-Par to confirm their hepatocellular phenotype and/or reticulin (Figure 4) to confirm hepatic plate expansion.

HCC in BDBs must be distinguished from other lesions that more typically involve the intra- and extrahepatic biliary tree and could potentially mimic it morphologically. By virtue of intraductal location, HCC must be distinguished from an invasive cholangiocarcinoma, which is far more frequent at this site. Although the intracytoplasmic mucin vacuoles that are typical of adenocarcinoma were not seen in any of our cases, cytoplasmic clearing (clear cell or steatohepatitic features) was present in some samples, and could potentially cause confusion with cholangiocarcinoma, as it did in one case. Tumor cell negativity for hepatocellular immunocytochemical markers as well as the absence of cytoplasmic bile pigment, should help with distinction between the two. Mixed HCC-cholangiocarcinoma, however, may be impossible to distinguish from pure HCC of brushings and may require examination of a larger sample. Other intraductal neoplasms that may show eosinophilic cells similar to those of HCC include intraductal papillary neoplasm of the bile duct (IPNB), particularly the oncocytic type, and oncocytic-type intraductal papillary mucinous neoplasm of pancreas (IOPN/IPMN-O), which may involve the biliary tree.[29-32] Both are characterized by papillary units lined by oncocytic cells with large nuclei, prominent nucleoli and little if any cytoplasmic mucin. These features may mimic the papillae seen in some of our cases. Endothelial wrapping, cytoplasmic bile pigment and positive hepatocellular markers would favor HCC. Well-differentiated neuroendocrine tumors (WDNETs) often metastasize to the liver or may involve bile ducts, and when oncocytic they may mimic HCC.[33,34] Unlike HCC, WDNETs have eccentric nuclei, salt-and-pepper chromatin and stain with neuroendocrine markers.[34] The distinction of well-differentiated HCC from normal hepatocytes which may also be sampled on BDB can be especially challenging. Identifying widened trabeculae with endothelial wrapping, nuclear pleomorphism and macronucleoli should favor HCC over benign hepatic parenchyma, but distinction may ultimately require biopsy.[13,14].

In summary, when HCC involves the biliary tree, it can lead to obstructive jaundice, ductal dilatation, and liver enzyme abnormalities, requiring ERCP and BDB which may inadvertently sample these tumors. Brushings are characterized by cells with naked nuclei or abundant granular cytoplasm, high nuclear-to-cytoplasmic ratio, large nuclei and macronucleoli. The classical cytologic features of HCC usually seen on aspiration (endothelial wrapping, widened trabeculae and cytoplasmic bile) are infrequent findings in this sampling modality. In order to avoid misdiagnosis cytopathologists should have a high index of suspicion when evaluating these samples, particularly in patients with concomitant liver lesions or known risk factors for HCC.

Legend to Figures

Figure 1: ThinPrep® slides **A - D**. **A.** There is a mixed (2-cell) population of benign ductal cells in honeycomb sheets (top center) and single and clustered malignant cells (center) with abundant granular cytoplasm, round nuclei and cherry red macronucleoli (Papanicolaou stain x 200). **B.** Tumor cells have high nuclear-to-cytoplasmic ratio, granular to clear cytoplasm and round hypochromatic and hyperchromatic nuclei with cherry red macronucleoli. (Papanicolaou stain x 600). **C.** Large hyperchromatic tumor cells with dense polygonal cytoplasm (left) and naked nuclei (right) (Papanicolaou stain x 600). **D.** 3-dimensional cluster with hypochromasia and markedly irregular nuclear membranes. This case was misdiagnosed as adenocarcinoma (Papanicolaou stain x 400).

Figure 2: **A**. ThinPrep® slides showing (**A**) a well-differentiated hepatocellular carcinoma with monotonous tumor cells with low nuclear-to-cytoplasmic ratio and clear cytoplasm (Papanicolaou stain x 400). Examples of poorly differentiated hepatocellular carcinoma showing marked (> 5-fold) anisonucleosis (**B**), multinucleated tumor giant cells (**C**) and cytoplasmic bile pigment (**D**) (Papanicolaou stain x 600).

Figure 3: Hepatocellular carcinoma with tumor cells arranged in widened trabeculae > 2 cells thick are shown in **A** (Hematoxylin & Eosin x 40) and **B** (Papanicolaou stain x 400). **C**.

Widened trabeculae are focally lined by flattened endothelial cells (arrow) (Papanicolaou stain x 400). **D.** Cell block showing well differentiated hepatocellular carcinoma (Hematoxylin & Eosin x 200).

Figure 4: **A, B.** Cell blocks show papillary units with central hyalinized cores lined by multilayered large eosinophilic to clear cells with relative monotony (Hematoxylin & Eosin stain x 200 – 400). **C.** Reticulin stain highlights widened trabeculae (x 200). Tumor cells are positive for arginase (**D**) and negative for Cytokeratin 7 while the benign ductal cells (upper right) are negative (**E**)(x 200). **F.** Hepatectomy specimen showing tumor growing as a circumscribed nodular intraductal mass that compresses the duct wall. Note the presence of dark blue DEB TACE spherules on the lower right (Hematoxylin & Eosin stain x 40).

References

1. Layfield LJ, Wax TD, Lee JG, Cotton PB. Accuracy and morphologic aspects of pancreatic and biliary duct brushings. *Acta Cytol.* 1995;39:11-18.
2. Logrono R, Kurtycz D, Molina C, Trivedi V, Wong J, Block K. Analysis of false-negative diagnoses on endoscopic brush cytology of biliary and pancreatic duct strictures. *Arch Pathol Lab Med.* 2000;124:387-392.
3. Choi WT, Swanson PE, Grieco VS, Wang D, Westerhoff M. The outcomes of "atypical" and "suspicious" bile duct brushings in the identification of pancreaticobiliary tumors: Follow-up analysis of surgical resection specimens. *Diagn Cytopathol.* 2015;43(11):885-891.
4. Okonkwo AM, De Frias DV, Gunn R, et al. Reclassification of "atypical" diagnoses in endoscopic retrograde cholangiopancreaticography-guided biliary brushings. *Acta Cytol.* 2003;47(3):435-442.
5. Volmar KE, Vollmer RT, Routbort MJ, Creager AJ. Pancreatic and bile duct brushing cytology in 1000 cases: review of findings and comparison of preparation methods. *Cancer.* 2006;108(4):231-238.
6. Hacihasanoglu E, Memis B, Pehlivanoglu B, et al. Factors Impacting the Performance Characteristics of Bile Duct Brushings: A Clinico-Cytopathologic Analysis of 253 Patients. *Arch Pathol Lab Med.* 2018;142(7):863-870.
7. Rupp M, Hawthorne CM, Ehya H. Brushing cytology in biliary tract obstruction. *Acta Cytol.* 1990;34(2):221-226.
8. Vadmal MS, Byrne-Semmelmeier S, Smilari TF, Hajdu SI. Biliary tract brush cytology. *Acta Cytol.* 2000;44(4):533-538.

9. Dusenbery D. Biliary stricture due to hepatocellular carcinoma: diagnosis by bile duct brushing cytology. *Diagn Cytopathol.* 1997;16(1):55-56.
10. Schmelzle M, Matthaei H, Lehwald N, et al. Extrahepatic intraductal ectopic hepatocellular carcinoma: bile duct filling defect. *Hepatobiliary Pancreat Dis Int.* 2009;8(6):650-652.
11. Fu LY, Mitchell KA, Cai G. Clear cell hepatocellular carcinoma diagnosed by bile duct brushing cytology. *Diagn Cytopathol.* 2016;44(2):147-151.
12. Torbenson MS, Ng IOL, Park YN, Roncalli M, Sakamato M. Hepatocellular carcinoma. In: Board WCoTE, ed. *WHO Classification of Tumours: Digestive System Tumours.* 5th ed. Lyon, France: IARC Press; 2019:229-239.
13. Das DK. Cytodiagnosis of hepatocellular carcinoma in fine-needle aspirates of the liver: its differentiation from reactive hepatocytes and metastatic adenocarcinoma. *Diagn Cytopathol.* 1999;21(6):370-377.
14. de Boer WB, Segal A, Frost FA, Sterrett GF. Cytodiagnosis of well differentiated hepatocellular carcinoma: can indeterminate diagnoses be reduced? *Cancer.* 1999;87(5):270-277.
15. Mallory TB, Castleman B, Parris BE. Case records of the Massachusetts General Hospital. *New England Journal of Medicine.* 1947;237:673-676.
16. Lin TY, Chen KM, Chen YR, Lin WS, Wang TH, Sung JL. Icteric type hepatoma. *Med Chir Dig.* 1975;4(5-6):267-270.
17. Okuda K. Clinical aspects of hepatocellular carcinoma: analysis of 134 cases. In: *Hepatocellular carcinoma.* New York: John Wiley; 1976:387-436.
18. Kew MC, Paterson AC. Unusual clinical presentations of hepatocellular carcinoma. *Trop Gastroenterol.* 1985;6(1):10-22.
19. Qin LX, Tang ZY. Hepatocellular carcinoma with obstructive jaundice: diagnosis, treatment and prognosis. *World J Gastroenterol.* 2003;9(3):385-391.
20. Narita R, Oto T, Mimura Y, et al. Biliary obstruction caused by intrabiliary transplantation from hepatocellular carcinoma. *J Gastroenterol.* 2002;37(1):55-58.

21. Buckmaster MJ, Schwartz RW, Carnahan GE, Strodel WE. Hepatocellular carcinoma embolus to the common hepatic duct with no detectable primary hepatic tumor. *Am Surg.* 1994;60(9):699-702.
22. Cho HG, Chung JP, Lee KS, et al. Extrahepatic bile duct hepatocellular carcinoma without primary hepatic parenchymal lesions--a case report. *Korean J Intern Med.* 1996;11(2):169-174.
23. Soyer P, Laissy JP, Bluemke DA, Sibert A, Menu Y. Bile duct involvement in hepatocellular carcinoma: MR demonstration. *Abdom Imaging.* 1995;20(2):118-121.
24. Saito M, Hige S, Takeda H, Tomaru U, Shibata M, Asaka M. Combined hepatocellular carcinoma and cholangiocarcinoma growing into the common bile duct. *J Gastroenterol.* 2001;36(12):842-847.
25. Dusenbery D. Combined hepatocellular-cholangiocarcinoma. Cytologic findings in four cases. *Acta Cytol.* 1997;41(3):903-909.
26. Jan YY, Chen MF. Obstructive jaundice secondary to hepatocellular carcinoma rupture into the common bile duct: choledochoscopic findings. *Hepatogastroenterology.* 1999;46(25):157-161.
27. Kojiro M, Kawabata K, Kawano Y, Shirai F, Takemoto N, Nakashima T. Hepatocellular carcinoma presenting as intrabile duct tumor growth: a clinicopathologic study of 24 cases. *Cancer.* 1982;49(10):2144-2147.
28. Kunz G, Jr., Chung J, Ali SZ. Hepatocellular carcinoma-fibrolamellar variant: cytopathology of an unusual case. *Diagn Cytopathol.* 2002;26(4):257-261.
29. Reddy K, Hooper K, Frost A, et al. Intraductal papillary carcinoma of common bile duct diagnosed by endoscopic ultrasound-guided fine-needle aspiration. *Endoscopy.* 2014;46 Suppl 1 UCTN:E248-249.
30. Reid MD, Stallworth CR, Lewis MM, et al. Cytopathologic diagnosis of oncocytic type intraductal papillary mucinous neoplasm: Criteria and clinical implications of accurate diagnosis. *Cancer Cytopathol.* 2016;124(2):122-134.
31. Jurczyk MF, Zhu B, Villa C, DeFrias D, Lin X. Cytomorphology of intraductal oncocytic papillary neoplasm of the liver. *Diagn Cytopathol.* 2014;42(10):895-898.

32. Bansal A, Thung SN, Zhu H, Schwartz M, Lewis S. Synchronous pancreatic adenocarcinoma and intrahepatic cholangiocarcinoma arising in the context of intraductal papillary neoplasms. *Clin Imaging.* 2016;40(5):897-901.

33. Volante M, La Rosa S, Castellano I, Finzi G, Capella C, Bussolati G. Clinico-pathological features of a series of 11 oncocytic endocrine tumours of the pancreas. *Virchows Arch.* 2006;448(5):545-551.

34. Chen S, Wang X, Lin J. Fine needle aspiration of oncocytic variants of pancreatic neuroendocrine tumor: a report of three misdiagnosed cases. *Acta Cytol.* 2014;58(2):131-137.

Table 1. Clinicopathologic Characteristics in 14 Patients

Patient	Age	Gender	Clinical History	Radiologic (CT/MRI) Findings	ERCP Findings	Cytology Diagnosis	Biopsy Diagnosis	FISH	Follow-up (mths)
1	59	M	Abdominal pain, fatigue, NASH cirrhosis	Tubular intraductal mass extending to porta hepatis, intrahepatic duct dilatation, cirrhosis Radiologic Dx HCC-ICC	Frond-like mass in CBD	Atypical cells	HCC-WD		DOD 6 mths
2	46	F	Nausea, vomiting, abdominal pain, ↑bili, AFP+LFTs	6.4cm liver mass w/ intrahepatic duct and CBD dilatation Radiologic Dx ICC	Dilated intrahepatic ducts w/ large intraductal mass	Atypical cells	HCC-WD		LTF/U 49 mths
3	60	M	Jaundice, HCV, ↑bili+LFTs	8.0 cm liver mass, cirrhosis, w/ intrahepatic duct dilatation Radiologic Dx HCC	Intrahepatic duct stricture	HCC	HCC-CC, PD	+	No resection DOD 2 mths
4	80	M	Hemochromatosis w/ h/o HCC, ↑bili, AFP+LFTs	2.2cm liver mass, portal vein thrombus Radiologic Dx HCC	Large mass in CBD/CHD	Negative	HCC-PD	-	No resection, DOD 46 mths
5	69	M	Jaundice, cirrhosis ↑bili+LFTs	No liver mass Radiologic Dx ICC	2.0cm mass seen in bile duct	HCC	HCC		DOD 7.5 mths
6	58	M	Asian, jaundice, HBV, family h/o HCC ↑bili, AFP,CA19-9+ LFTs	11.3cm liver mass + 3.6cm porta hepatis/CBD junction mass; portal vein thrombus; Radiologic Dx HCC	Clots in hepatic duct	HCC-PD	No	+	DOD 1 mth
7	55	M	AA, jaundice, HCV, cirrhosis, ↑bili, AFP+ LFTs	Multiple liver masses, largest 5cm, cirrhosis Radiologic Dx HCC	No mass or stricture	ICC	HCC		Tumor embolization, resection, TX; T3bN0; Adrenal mets at 4 yrs AWD, 48 mths
8	45	M	Asian, jaundice bacterial sepsis, HAV, HBV, EBV, ↑bili, AFP+ LFTs	8.9 cm liver mass w/ intrahepatic duct dilation Radiologic Dx HCC vs ICC	Debris in intrahepatic ducts and CBD	HCC	HCC-WD		No Rx AWD 0.2 mths
9	77	M	Jaundice, ↑bili+LFTs	13cm liver mass Radiologic Dx ICC	Stricture in intrahepatic ducts, no dilatation	HCC	HCC		chemoRx Died of intrahepatic hemorrhage 2 mths

10	60	M	HCV, N bili, ↑LFTs	6.6cm tumor at porta hepatis w/ obstruction of intrahepatic ducts; Radiologic Dx ICC	No mass or stricture	Favor HCC	No	Tumor embolization DOD 2 mths
11	57	F	Jaundice, ↑bili, LFTs	6.0cm liver mass w/ diffuse intrahepatic duct dilatation Radiologic Dx ICC	Stricture in hilum w/ bilateral hepatic duct obstruction	HCC	HCC	No Rx 5 mths, DOD
12	76	M	↑bili, LFTs	7.4 cm liver mass with w/ intrahepatic duct dilation, portal vein thrombus; Radiologic Dx HCC	Mass in bile duct	HCC	HCC-WD	chemoRx DOD, 1 mth
13	71	M	h/o HCC 2° to ASH cirrhosis post-TX w/ jaundice, ↑bili, CEA, CA19-9, LFTs	Multiple liver masses, largest 3.5cm, right intrahepatic duct dilatation Radiologic Dx HCC	Stricture in right hepatic duct	HCC	No	No Rx DOD 30 mths,
14	22	M	h/o resected Fibrolamellar HCC, ↑bili + LFTs	2.2 cm nodule at resection margin and intrahepatic bile duct dilatation near neo hepatis portis and stricture Radiologic Dx HCC	Not available	Fibrolamellar HCC	Fibrolamellar HCC	Wedge resection AWD, 39ths

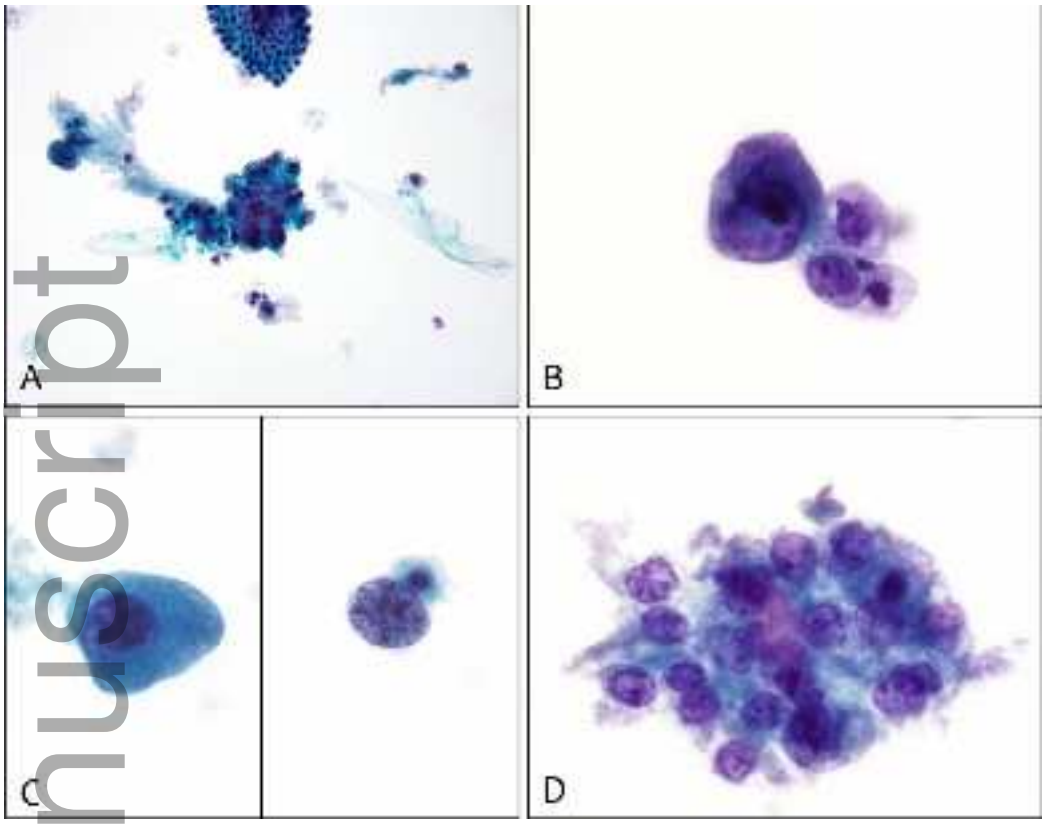
ERCP, endoscopic retrograde cholangiopancreatogram; FISH, fluorescence in-situ hybridization; HCC, hepatocellular carcinoma; WD, well differentiated; PD, poorly differentiated; mths, months; ICC, intrahepatic cholangiocarcinoma; CBD, common bile duct; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; EBV, Epstein Barr virus; TX, transplant; ASH, alcoholic steatohepatitis; NASH, non-alcoholic steatohepatitis; AA, African American; ↑bili, increased serum bilirubin; AFP, alpha fetoprotein; LFT, liver function tests; Dx, diagnosis; DOD, died of disease; AWD, alive with disease; LTF/U, lost to follow-up.

Table 2. Cytologic Findings in 17 Bile Duct Brushing Samples

Feature	1	2	3	4	5*	6*	7	8	9*	10*	11	12	13	14	15	16*	17*	Total (%)
Hypercellularity			+	+				+	+	+		+			+			7 (41%)
Single intact cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	17 (100%)
Atypical naked nuclei	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	17 (100%)
3-D clusters	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	17 (100%)
Granular cytoplasm	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	17 (100%)
Prominent nucleoli	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	16 (94%)
Widened trabeculae									+	+	+		+	+	+			6 (35%)

High N:C	+	+	+	+				+	+	+	+	+	+	+	+	+							13 (77%)
Necrosis				+	+	+	+	+								+	+	+					8 (47%)
2-cell population				+				+						+	+	+	+	+	+	+			8 (47%)
Hypercellularity			+	+				+	+	+			+					+					7 (41%)
Clear/microvesicular cytoplasm			+		+	+		+	+	+													6 (35%)
Cytoplasmic bile				+			+										+	+	+	+			6 (35%)
Nuclear irregularity			+		+	+			+	+			+										6 (35%)
Anisonucleosis				+	+			+					+						+	+			6 (35%)
Papillary groups				+	+	+	+			+													5 (29%)
Multinucleated tumor cells				+		+	+		+														4 (24%)
Endothelial wrapping				+				+					+					+					4 (24%)
Ancillary studies (IHC/Reticulin)				+	+	+	+	+	+	+	+			+	+								10 (59%)

*, 5 and 6 are same patient; 9 and 10, same patient; 16 and 17, same patient; 3-D, 3-dimensional; high N/C, nuclear to cytoplasmic ratio; IHC, immunohistochemical stains including Hep-Par, glypican 3 and arginase.



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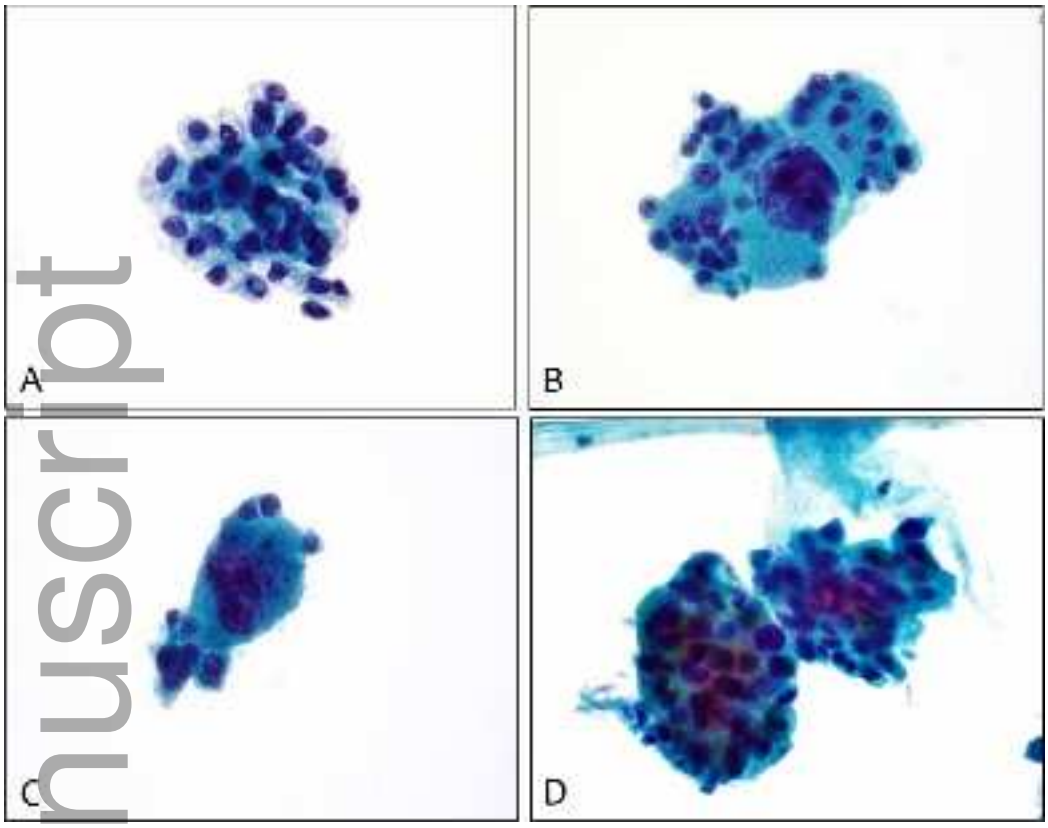

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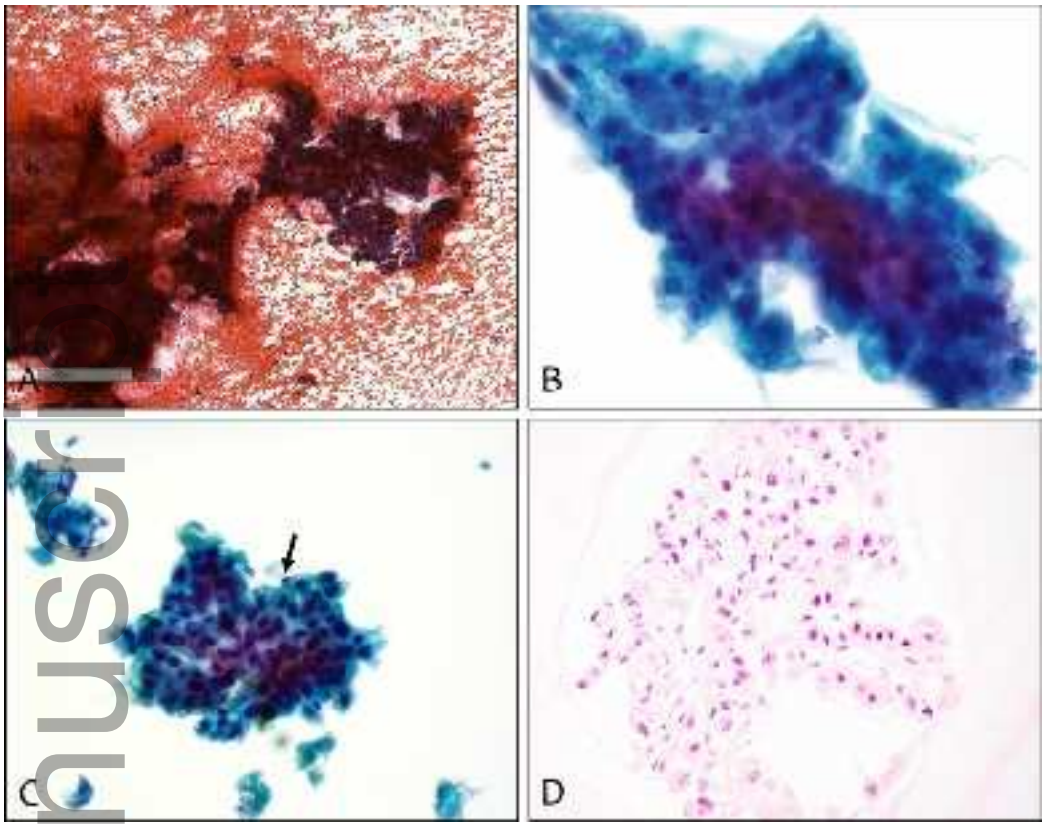

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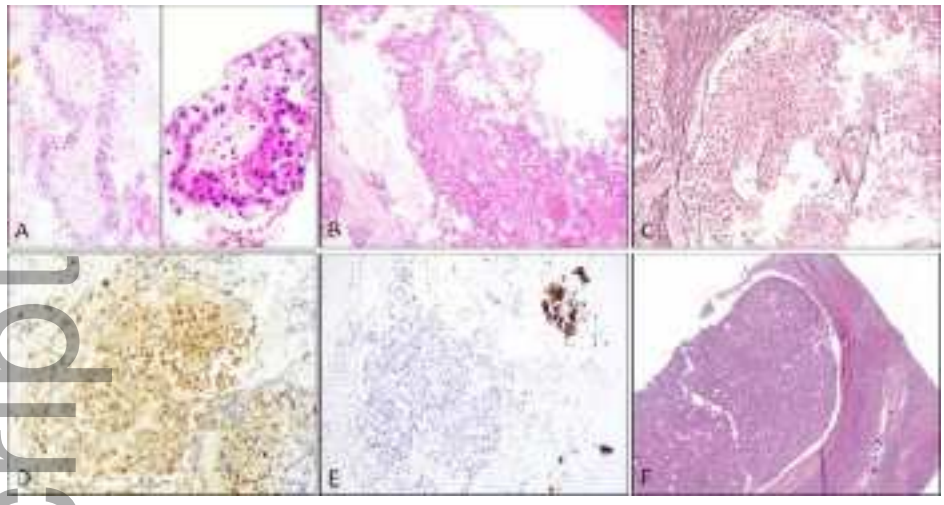

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