

Hepatocellular Carcinoma Diagnosed in Bile Cytology: A Case Report and Review of the Literature

Mariana Zaarour¹, Gael Roth^{2,3,4}, Pierre-Yves Eyraud³, Nathalie Sturm^{1,2,5} and Diane Giovannini^{1*}

¹Department of Pathology and Cytology, Grenoble-Alpes University Hospital, Grenoble, France

²Grenoble-Alpes University, Grenoble, France

³Hepatogastroenterology and Digestive Oncology Clinic, Grenoble-Alpes University Hospital, Grenoble, France

⁴Institute for Advanced Biosciences - CNRS UMR/INSERM, Grenoble, France

⁵T-RAIG, TIMC-IMAG, Grenoble-Alpes University, CNRS UMR, La Tronche, France

Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy with a high mortality rate. The gold standard for making the diagnosis is liver biopsy. However, this technique can sometimes be risky and contraindicated. Endoscopic retrograde cholangiopancreatography is a largely used therapeutic procedure in hepatobiliary malignancies in case of jaundice. The latter offers also an easy access to bile samples suitable for cytological examination. In addition to previously reported cases of HCC diagnosed by bile duct brushings, we are reporting the first case of endobiliary HCC diagnosed by bile aspiration. We emphasize this rare and non-invasive cytological technique presenting advantages such as cell block preparation and immunohistochemistry.

Keywords: Cytology • Bile aspiration • Hepatocellular carcinoma • Cell block

Introduction

Hepatocellular carcinoma (HCC) is listed in recent data as the fifth most common cause of cancer with 750,000 new cases of HCC per year and the second cancer-related death worldwide [1]. It is defined by the World Health Organization as primary malignancy of the liver composed of epithelial cells showing hepatocellular differentiation. The main risk factors include viral hepatitis B and C, alcoholic and non-alcoholic steatohepatitis among others. Liver biopsy remains the gold standard for diagnosing liver diseases and more specifically HCC [2-4]. In biliary specimens, cholangiocarcinoma is the usual type of carcinoma diagnosed. Few cases of HCC were reported in bile duct brushing (BDB) series [5-9], however, none was described in bile duct aspiration cohorts [10]. To our knowledge we report the first case of HCC diagnosed in extra-hepatic bile cytology confirmed by liver biopsy.

Case Presentation

Clinical findings

A 54 years old male patient was admitted to the emergency department for weakness, jaundice, pruritis and 2 weeks history of abdominal pain. In his past medical history, he had liver cirrhosis, oxygen dependent chronic obstructive pulmonary disease, chronic renal failure and ischemic cardiomyopathy. On physical examination, he was icteric and had abdominal tenderness. Liver blood tests showed cholestasis and cytolysis with elevated conjugated bilirubine, gamma-glutamyl transferase and transaminase associated with

high alpha-fetoprotein level. Abdominal ultrasound showed a multi nodular liver parenchyma with bilateral intra hepatic bile duct dilation (Figure 1A). Due to acute renal failure, abdominal computed tomography scan (CT scan) was done without contrast injection (Figure 1B). Results were no conclusive concerning a suspicious intra hepatic lesion (7.9 × 6.4 cm) along with signs of chronic liver injury. The patient underwent an endoscopic retrograde cholangiopancreatography (ERCP) that showed left and right hepatic duct stenosis. Thick purulent material was evacuated from narrowing site and bile was collected before and after BDB and sent for cytological examination.

Bile cytopathologic findings

Bile aspiration specimen was performed as previously described by our team [11,12]. Bile smear was hypercellular, showing both highly isolated malignant cells and tridimensional clusters. The tumor cells were large, polygonal with an abundant granular cytoplasm, an enlarged nucleus and prominent nucleoli along with multiple naked nuclei. The presence of cytoplasmic bile pigments was observed. The background was rich in neutrophils with some benign appearing bile duct epithelium (Figure 2). Hematoxylin-eosin-safran staining on cell block (CB) showed histologically sheets malignant hepatocytes with few intracytoplasmic bile pigments and Mallory-Denk bodies (Figure 3). Immunohistochemical study performed on CB confirmed the presence of hepatocellular differentiation with Hepatocyte and Arginase antibodies positivity (Figure 4A) and the malignant nature with Glypican 3 positivity (Figure 4B).

BDB showed a similar cytology pattern consisting of large malignant cells

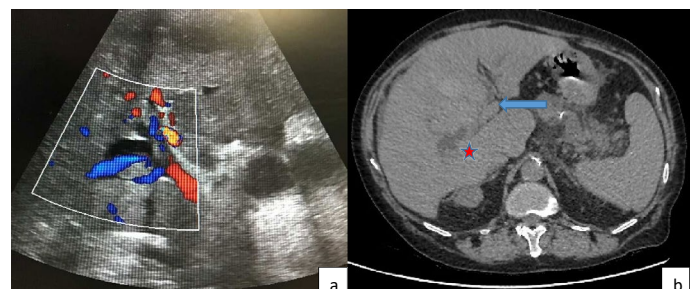


Figure 1. (a) Abdominal ultrasound with doppler and (b) abdominal CT-scan without enhancement showing a central intrahepatic tumor invading the bile duct convergence (blue arrow) and the portal vein (asterix).

***Address for Correspondence:** Diane Giovannini, Department of Pathology and Cytology, Grenoble-Alpes University Hospital, Grenoble, France, Tel: +04767664z4, E-mail: dgiovannini@chu-grenoble.fr

Copyright: © 2022 Zaarour M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Date of Submission: 29 Aug 2022, Manuscript No. jct-22-73144; Editor assigned: 01 Sep 2022, Pre QC No. P-73144; Reviewed: 12 Sep 2022, QC No. Q-73144; Revised: 19 Sep 2022, Manuscript No. R-73144; Published: 26 Sep 2022, DOI: 10.37421/1948-5956.2022.14.544

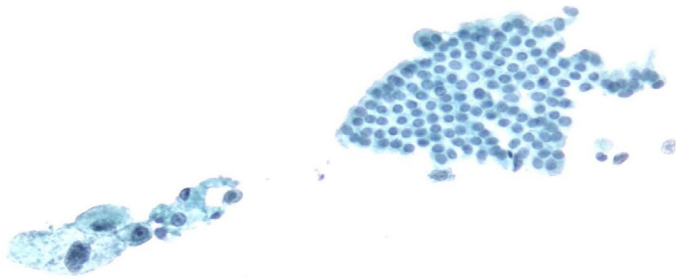


Figure 2. Bile samples obtained by aspiration showing malignant hepatocytes on the left and normal duct epithelium in an honeycomb arrangement on the right. Papanicolaou stain (X10).

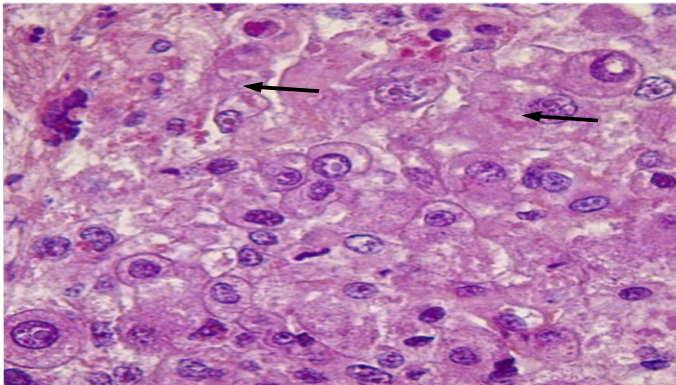


Figure 3. Bile aspiration showing polygonal cells with large nuclei, prominent nucleoli and cytoplasmic Mallory-Denk bodies (black arrows), cell block stained by hematoxylin-eosine-safran (X40).

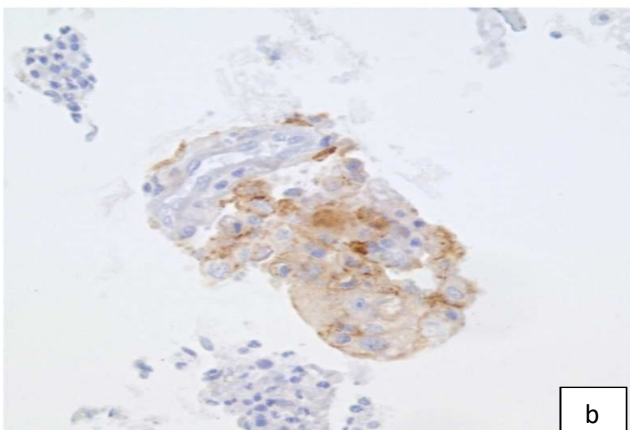
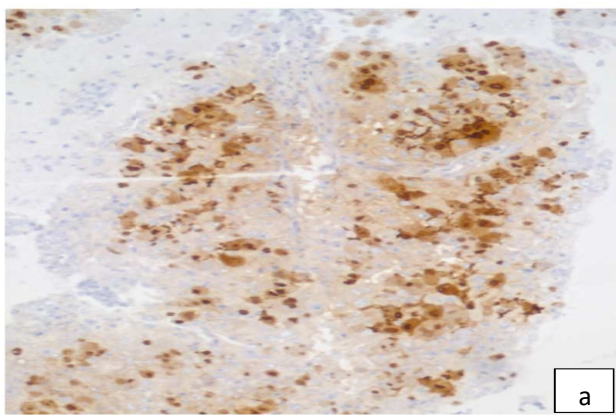


Figure 4. Immunohistochemistry on bile aspiration cell block. (a) Arginase immunolabelling of tumoral hepatocytes at low power field (X10) and (b) Glypican 3 immunolabelling of tumoral hepatocytes at intermediate power field (X20).

with hepatoid morphology, some with crushing artefacts, on an inflammatory background and normal appearing duct epithelium (Figure 5).

After patient stabilization and obstruction relieve, a core needle biopsy was done for confirmation. Microscopically, it confirmed liver cirrhosis and showed foci of moderately differentiated HCC, Edmondson and Steiner's grade 2, made up by medium to large tumor cells with eosinophilic cytoplasm and occasionally Mallory-Denk bodies (Figure 6). The nuclei were round to oval with moderate anisocaryosis and prominent nucleoli. The cells formed large trabeculae and expressed Arginase and Hepatocyte antigens on immunohistochemical analysis.

Due to the fact of advanced stage disease, the oncologists decided that the patient was not a candidate for surgery, chemotherapy or radiotherapy, and he only received supportive management. Due to multiorgan failure, the patient died 9 days after the diagnosis of HCC.

Discussion

The classic way for diagnosing HCC requires imaging and/or liver biopsy which represents the gold standard for the diagnosis [13]. However, in case of tumor protruding into the bile ducts, the diagnosis of HCC may also be made by cytology obtained after ERCP and we are reporting the first case of intra hepatic HCC invading the bile ducts diagnosed by bile aspiration cytology with CB preparation.

Based on literature review, only 17 diagnosis of HCC with cytology methods were previously reported: 16 cases after BDB and one case after

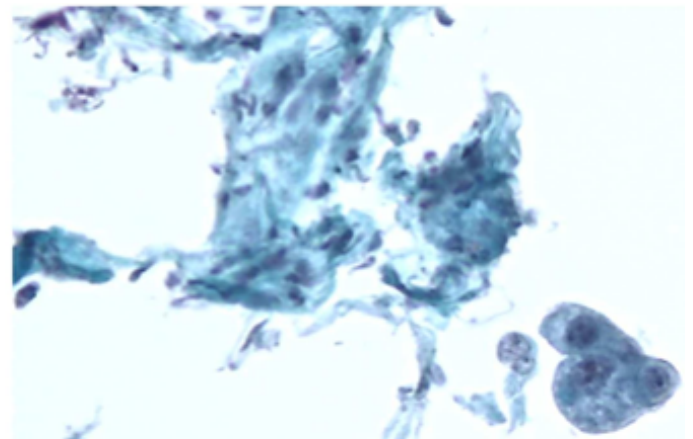


Figure 5. Bile duct brushing showing polygonal cells with large nuclei and visible nucleoli (bottom right), some of them were altered by crushing artefacts seen on the left. Papanicolaou stain (X20).

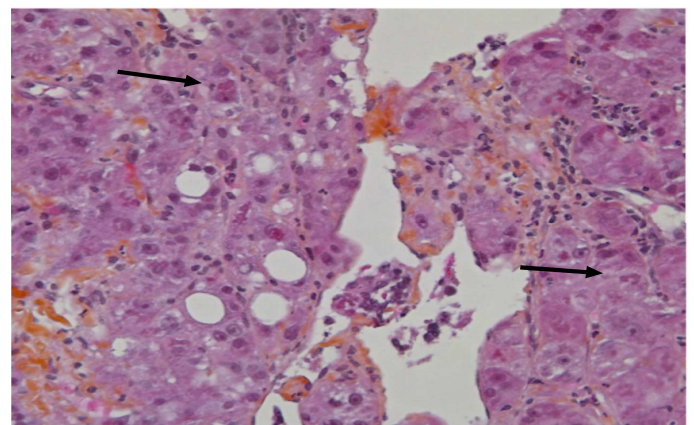


Figure 6. Transperietal core needle liver biopsy showing moderately differentiated HCC made up of polygonal cells with large nuclei, prominent nucleoli and Mallory-Denk bodies (black arrow). Hematoxylin-eosine-safran stain (X10).

Table 1. Literature review of HCC diagnosed from cytology specimens.

Authors	Number of Cases	Single (S)/Multiple (M)	Intrahepatic(I)/Hilar (H)	Cytology Technique	Histology	Diagnosis
Fu, LY [4]	1	S	I	BDB	Surgical specimen	HCC (clear cell)
Bhattarai, S [5]	14	S + M	H	BDB	Biopsies	11 HCC (WD,PD, Fibrolamellar,NOS)
Kunz, G [6]	1	S	H	BDB+FNA	Surgical specimen	HCC (Fibrolamellar variant)
Dusenbery D [7]	1	S	I	BDB	Biopsy	HCC
Schmelzle M [8]	1	S	I	BDB	Biopsy	HCC, ectopic
Our case	1	S	I	BA + BDB	Biopsy	HCC (MD)

BDB and fine needle aspiration (FNA) [5-9], (Table 1). Fifteen were confirmed after histology (core needle biopsy/ surgical resection).

Even though liver biopsy is the gold standard for HCC diagnosis, it may be a risky technique in some cases with variable complications influenced by several factors such as coagulation status, operator experience, presence of ascites. The most frequent complication is the post-operative pain, reaching up to 86% of cases. Other complications are transient bacteremia (6-14%) and hemorrhage (1%) [14].

In case of biliary obstruction, the ERCP represents a diagnostic and therapeutic procedure at the same time which allows bile ducts drainage by dilation and stent placement in case of stenosis and gives access to intraductal samples. In a large study, complication rate did not exceed 7%, with the most of them being benign [15]. The most serious complication is infectious cholangitis observed in 1.4%, with 7.85% mortality rate. Bile duct drainage has been for so long as a debatable option for infectious risk reduction [16,17]. Haemorrhage (2%), severe bleeding and perforation are extremely rare. Acute pancreatitis is associated with multiple contrast injection during the procedure [18-21].

In case of jaundice requiring an ERCP, intraductal samples should be systematically performed as they do not increase the morbidity of the procedure and they may provide a histopathological diagnosis. In these specific conditions, bile aspiration might be an alternative to core needle biopsy even though diagnostic performances, as well as the histological HCC subtypes determination, are unknown.

Besides, bile aspiration samples obtained during ERCP are of good quality for cytological analysis, if they are well conditioned. Unlike samples obtained by basic brushing, there do not present crushing artefacts and the realization of CB open the way to additional immunohistochemical and molecular studies [12].

Conclusion

In case of HCC obstructing bile ducts, bile samples obtained by aspiration during ERCP may represent a simple and safe way to obtain good quality samples allowing a diagnosis without increasing the invasiveness of the technique. In this clinical condition, it might be an alternative to the classical liver biopsy whose infectious risk is increased in case of bile duct obstruction. Moreover, whereas brushing samples quality is usually low, bile aspiration provides non altered cells, which is compatible with morphological, immunohistochemical and molecular analyses.

References

- Ferlay, Jacques, Isabelle Soerjomataram, Rajesh Dikshit, and Sultan Eser, et al. "Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012." *Int J Cancer* 136 (2015): E359-E386.
- European Association for the Study of the Liver. "EASL clinical practice guidelines: Management of hepatocellular carcinoma." *J Hepatol* 69 (2018): 182-236.
- Blanc, Jean Frédéric, Audrey Debaillon-Vesque, Gaël Roth, and Jean Claude Barbare, et al. "Hepatocellular carcinoma: French Intergroup Clinical Practice Guidelines for diagnosis, treatment and follow-up (SNFGE, FFCD, GERCOR, UNICANCER, SFCD, SFED, SFRO, AFEF, SIAD, SFR/FRI)." *Clin Res Hepatol Gastroenterol* 45 (2021): 101590.
- Pandey, Nivedita, Gilles J. Hoilat, and Savio John. "Liver biopsy." (2017).
- Fu, Li-Ying, Kisha A. Mitchell, and Guoiping Cai. "Clear cell hepatocellular carcinoma diagnosed by bile duct brushing cytology." *Diagn Cytopathol* 44 (2016): 147-151.
- Bhattarai, Shristi, Rondell P. Graham, Carlisle S. Sigel, and Jiaqi Shi, et al. "Bile duct involvement by hepatocellular carcinoma: A rare occurrence and poor prognostic indicator in bile duct brushing samples." *Cancer Cytopathol* 127 (2019): 691-699.
- Kunz Jr, George, Jeffrey Chung, and Syed Z. Ali. "Hepatocellular carcinoma fibrolamellar variant: Cytopathology of an unusual case." *Diagn Cytopathol* 26 (2002): 257-261.
- Dusenbery, David. "Biliary stricture due to hepatocellular carcinoma: diagnosis by bile duct brushing cytology." *Diagn Cytopathol* 16 (1997): 55-56.
- Curcio, Gabriele, Mario Traina, Filippo Mocciano, and Rosa Liotta, et al. "Intraductal aspiration: a promising new tissue-sampling technique for the diagnosis of suspected malignant biliary strictures." *Gastrointest Endosc* 75 (2012): 798-804.
- Schmelzle, Moritz, Hanno Matthaei, Nadja Lehwald, and Andreas Raffel, et al. "Extrahepatic intraductal ectopic hepatocellular carcinoma: bile duct filling defect." *Hepatobiliary Pancreat Dis Int* 8 (2009): 650-652.
- Giovannini, Diane, Adeline Bailly, Arnaud Seigneurin, and Michèle Fior Gozlan, et al. "Biliary cytology: A diagnostic tree for adenocarcinoma based on a cohort of 135 patients with endoscopic retrograde cholangiopancreatography for stenosis of the extrahepatic bile duct." *Cancer Cytopathol* 130 (2022): 433-442.
- Fior Gozlan, Michèle, Diane Giovannini, Maud Rabeyrin, and Anne Mc Leer Florin, et al. "Monocentric study of bile aspiration associated with biliary brushing performed during endoscopic retrograde cholangiopancreatography in 239 patients with symptomatic biliary stricture." *Cancer Cytopathol* 124 (2016): 330-339.
- Bialecki, Eldad S and Adrian M. Di Bisceglie. "Diagnosis of hepatocellular carcinoma." *HPB* 7 (2005): 26-34.
- Machado, Norman Oneil. "Complications of liver biopsy-risk factors, management and recommendations." *Liver Biopsy* (2011): 393-404.
- Szary, Nicholas M and Firas H. Al-Kawas. "Complications of endoscopic retrograde cholangiopancreatography: How to avoid and manage them." *Gastroenterol Hepatol* 9 (2013): 496.
- Cotton, Peter B., Donald A. Garrow, Joseph Gallagher, and Joseph Romagnuolo. "Risk factors for complications after ERCP: A multivariate analysis of 11,497 procedures over 12 years." *Gastrointest Endosc* 70 (2009): 80-88.
- Naitoh, Itaru, Hirota Ohara, Takahiro Nakazawa, and Tomoaki Ando, et al. "Unilateral vs. bilateral endoscopic metal stenting for malignant hilar biliary obstruction." *J Gastroenterol Hepatol* 24 (2009): 552-557.
- Loperfido, Silvano, Giampaolo Angelini, Giorgio Benedetti, and Fausto Chilovi, et al. "Major early complications from diagnostic and therapeutic ERCP: A prospective multicenter study." *Gastrointest Endosc* 48 (1998): 1-10.
- Freeman, Martin L. "Adverse outcomes of ERCP." *Gastrointest Endosc* 56 (2002): S273-S282.
- Cotton, P.B., G. Lehman, J. Vennes and J.E. Geenen, et al. "Endoscopic sphincterotomy complications and their management: An attempt at consensus." *Gastrointest Endosc* 37 (1991): 383-393.
- Barthel, M., N. Lesavre, A. Desjeux and M. Gasmi, et al. "Complications of endoscopic sphincterotomy: Results from a single tertiary referral center." *Endoscopy* 34 (2002): 991-997.

How to cite this article: Zaarour, Mariana, Gael Roth, Pierre-Yves Eyraud and Nathalie Sturm, et al. "Hepatocellular Carcinoma Diagnosed in Bile Cytology: A Case Report and Review of the Literature." *J Cancer Sci Ther* 14 (2022): 544.