

## **The Diagnostic Value of Immunohistochemistry in the Diagnosis of Primary and Secondary Hepatic Carcinomas**

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*Abstract.* Metastatic adenocarcinoma to the liver from an unidentified primary tumor site is a common diagnostic problem. The present study included 41 cases of histologically diagnosed liver biopsies including hepatocellular carcinoma, cholangiocarcinoma and metastatic carcinoma in the liver. All cases were stained immunohistochemically with Cytokeratin 18, 7, and 20, Carbohydrate Antigen 19-9 and Alpha-fetoprotein to evaluate their usefulness in differentiating these tumor entities. Cytokeratin 18 was positive in 87.5% of hepatocellular carcinoma; all cases of cholangiocarcinoma, and metastatic carcinoma. Cytokeratin 7+ve/Cytokeratin 20-ve pattern was identified in 12.5% of hepatocellular carcinoma, 100% of cholangiocarcinoma, one metastatic pancreatic carcinoma, and all metastatic gastric carcinomas (100%). On the other hand, Cytokeratin 7-ve/Cytokeratin 20+ve were identified in colorectal carcinoma metastatic to the liver. CA19-9 showed positive immunoreactivity in all studied cases. AFP positive immunostaining was identified in 43.7% of hepatocellular carcinoma while it was negative in all other tumors. It was concluded that Cytokeratin 18 was of no benefit in the differential diagnosis of primary hepatic carcinoma and metastatic cases from any site. Cytokeratin 7 and CA19-9 positive staining can exclude a diagnosis of hepatocellular carcinoma, but cannot discriminate between metastatic carcinoma (from stomach, pancreaticobiliary origin) and cholangiocarcinoma. The Cytokeratin 20+/Cytokeratin 7-ve phenotype indicates metastatic intestinal adenocarcinoma, most often from the colon or rectum.

*Keywords:* Hepatocellular carcinoma, Cholangiocarcinoma, Metastatic carcinoma, Immunohistochemistry, Cytokeratins, CA-19-9, Alpha-fetoprotein.

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## Introduction

Hepatocellular carcinoma (HCC) is known for its histomorphologic heterogeneity. The comparative morphologic evaluation of HCC and their mimics is often a challenging issue. Some of these diagnostic challenges can be attributed to: a) The variety of neoplasms that can arise from the hepatic cells, b) The liver is a target for metastases that can mimic variants primary hepatocellular carcinoma, and c) The limitations of serum Alpha-fetoprotein (AFP) in the distinction of a poorly differentiated HCC from cholangiocarcinoma (CC) and metastatic carcinomas.

Various immunohistochemical markers have been advocated for the identification of these tumors that include  $\alpha$ -1-antitrypsin, Carcinoembryonic Antigen (CEA), factor XIIIa, ferritin, and albumin. However, their ability to distinguish HCC from other malignancies has been limited. Anti-AFP and anti-polyclonal carcinoembryonic antigen (anti-CEA) antibodies are traditionally used as positive markers for HCC<sup>[1]</sup>. The sensitivity of AFP is low, ranging from 17-61.5%<sup>[1-4]</sup>. AFP may also infrequently stain other types of carcinomas, including gastric, colonic, and CC<sup>[1-3]</sup>. The characteristic canalicular staining with anti-CEA is reported to be quite specific for hepatocellular differentiation, being reported in 15-80% of HCC. However, it is often difficult to be interpret, and it was reported to be positive in other gastrointestinal carcinomas, thus limiting their use in the diagnosis of hepatocellular carcinoma<sup>[4-7]</sup>.

## Aim

To evaluate the usefulness of immunohistochemical staining for cytokeratins (CK) 18, 7, and 20; together with CA19-9 and Alfa-fetoprotein in the differential diagnosis of hepatocellular carcinoma from cholangiocarcinoma, and metastatic gastrointestinal carcinoma.

## Materials and Methods

The present study included 41 cases of histologically diagnosed needle and excision biopsies obtained from the liver. Tumor tissue blocks and clinical data of the cases were collected from the files of the Department of Pathology at the King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia from period of 2002 to 2007. Cases

were histologically diagnosed as: HCC ( $n = 16$ ), CC ( $n = 6$ ) and metastatic carcinoma to the liver ( $n = 19$ ). All cases had correspondingly typical clinical findings. Diagnoses were confirmed by imaging techniques, at laparotomy, or by using tumor serum markers as AFP and carcinoembryonic antigen. Nineteen cases of metastatic adenocarcinoma were driven from; the colon ( $n = 9$  cases), pancreatic carcinoma ( $n = 2$  cases), extra hepatic biliary carcinoma ( $n = 4$  cases), and stomach ( $n = 4$  cases).

Five-micrometer sections from selected tumor blocks were mounted on 3-aminopropyltriethoxysilane coated (Sigma, St. Louis, MO USA) slides and were deparaffinized in xylene, rehydrated in graded alcohols, plus rinsed in 0.05 m Tris-buffered saline (TBS). Sections were boiled in 10 mm citrate buffer for antigen retrieval, at pH 6.0. Endogenous peroxidase was blocked with aqueous 0.3%  $H_2O_2$  for 15 min. An avidin–biotin–peroxidase method was employed as described in manufacturers' kit manual. The sections were incubated in 5% normal rabbit serum followed by one hour incubation in CK 18 (1:100, Dako, Carpentina), CA19-9 (1:50, LabVision, Neo Markers), CK 7 (1:100, Dako, Carpentina), monoclonal antibody to Alfa-fetoprotein (1:100, Dako, Carpentina), and CK 20 (1:50 dilution, Dako, Carpentina). As negative control, the primary antibody was replaced by TBS, and the appropriate normal areas in the sections served as positive controls. Positive immunoreactivity was defined as more than 20% of cells staining with the proper pattern of reactivity. Immunopositivity to CK18, CK7, CK20 and AFP appeared as brown cytoplasmic staining of tumor cells, while positivity to CA19-9 appeared as luminal staining of tumor cells.

## Results

Immunohistochemical results were collected and described in Table 1.

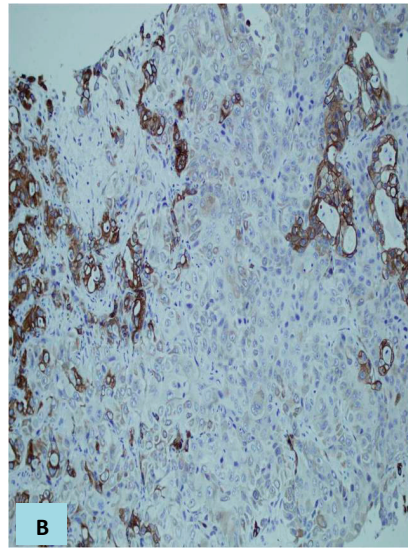
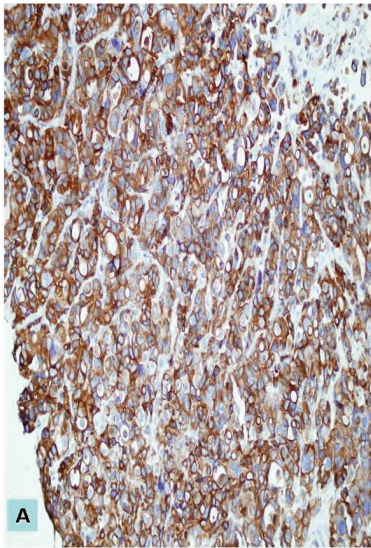
### *Hepatocellular carcinoma (HCC)*

Immunohistochemical staining of HCC cases showed positive immunoreactivity for antibodies to AFP in 7/16 (43.7%) of cases. Positive immunoreactivity for antibodies to CK 18 in 14/16 HCC (87.5%) (Fig. 1a). CK7 showed positive immunoreactivity in 2/16 (12.5%) of HCC. Staining appeared as focal positivity to CK 7 in certain

tumor areas while it was negative in adjacent areas (Fig. 1b). CK 7 staining usually was distinct in normal intrahepatic bile duct epithelium which was often proliferated and intermingled with the tumor cells, thus, caused some difficulties in the interpretation. HCCs were negative to CK20, and CA19-9.

**Table 1. Immunohistochemical profile of HCC, CC and metastatic GIT carcinoma.**

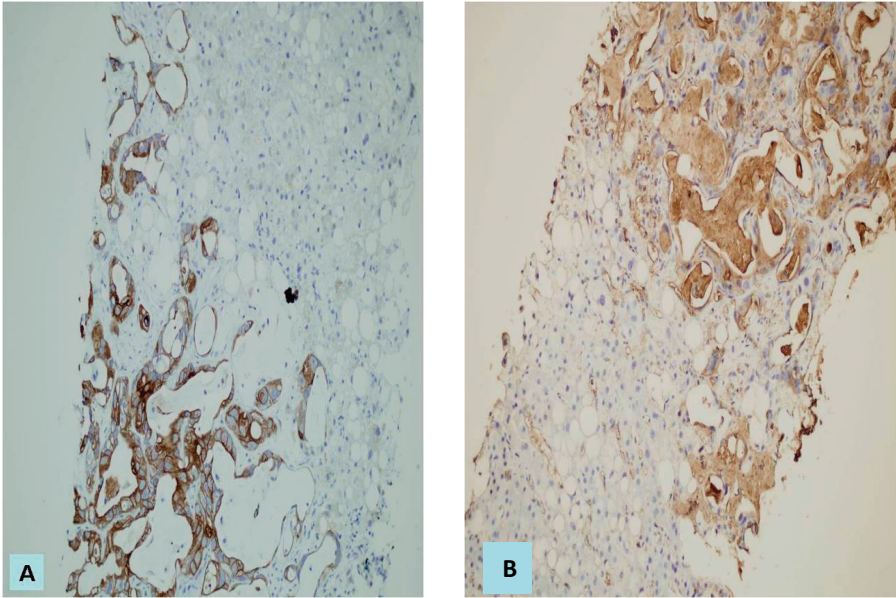
Type of Carcinomas		Number of Cases (n=41)	Number and Percentage of Immunohistochemical Positive Cases (%)				
			CK18	CA19-9	CK7	CK20	AFP
1ry hepatic carcinoma	HCC	16	N = 14 (87.5%)	-ve	N = 2 (12.5%)	-ve	N = 7 (43.7%)
	CC	6	N = 6 (100%)	N = 6 (100%)	N = 6 (100%)	-ve	-ve
2ry hepatic carcinoma	Colon	9	9 (100%)	9 (100%)	-ve	9 (100%)	-ve
	Pancreas	2	2 (100%)	2 (100%)	1 (50%)	-ve	-ve
	Biliary tract	4	4 (100%)	4 (100%)	4 (100%)	-ve	-ve
	Stomach	4	4 (100%)	2 (50%)	4 (100%)	-ve	-ve



**Fig. 1. Immunohistochemical staining of hepatocellular carcinoma: A) Hepatocellular carcinoma showing strong cytoplasmic staining to CK 18. (ABC, X 200). B) Mixed hepatocellular-cholangiocarcinoma showing negative staining to CK 7 in classic trabecular/ sinusoidal areas while adjacent neoplastic tissue displaying glandular arrangement are positive to CK7 (ABC, X 100).**

### ***Cholangiocarcinoma (CC)***

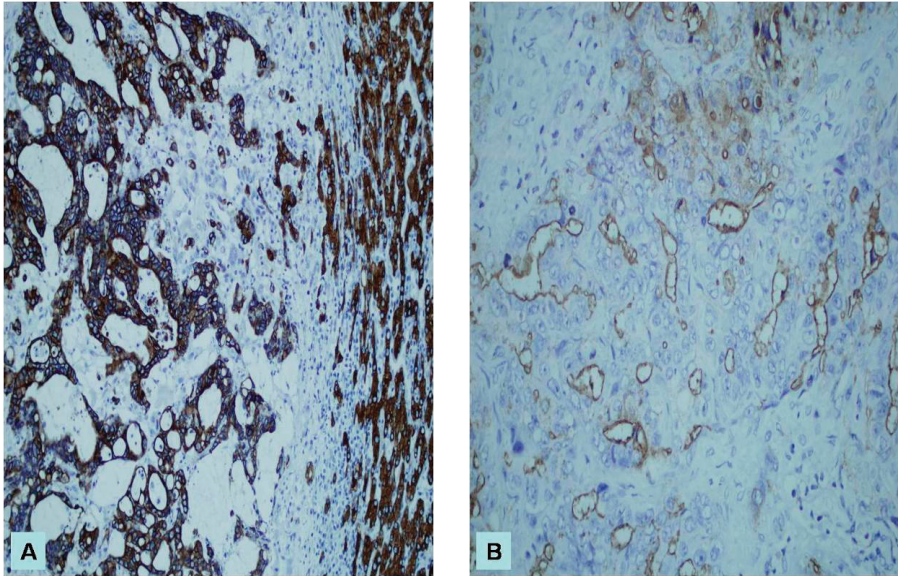
Immunohistochemical staining of CC cases showed positive immunoreactivity in all cases to CK 18, CK7, and CA19-9. On the other hand, all cases were negative to antibodies to AFP and CK20 (Fig. 2).



**Fig. 2. Immunohistochemical staining of cholangiocarcinoma: A) Cholangiocarcinoma showing positive cytoplasmic stain for CK-7. Note; negative intervening hepatocytes (ABC, X200). B) Cholangiocarcinoma showing positive cytoplasmic stain for CA19-9. Note negative adjacent hepatocytes (ABC, X100).**

### ***Metastatic Carcinomas***

The metastatic carcinomas to the liver from a primary in the colon showed strong immunoreactivity to CK 20, CA19-9, and CK18 (9/9) while they were negative to AFP and CK7. Metastatic carcinomas from a primary tumor in the pancreaticobiliary region showed negative reactivity to AFP, and a positive reaction to CA19-9 in 6/6 (100%). CK7+ve / CK20-ve pattern was demonstrated in 1/2 carcinoma of pancreatic origin and in 4/4 extra hepatic biliary carcinoma, whereas one pancreatic carcinoma showed CK7-ve/CK20-ve pattern. Metastatic gastric carcinomas were negative to AFP, and CK20 in all cases, while (4/4) were positive to CK7, CK18 and (2/4) were positive to CA19-9 (Fig. 3).



**Fig. 3. Immunohistochemical staining of metastatic carcinoma: A) Metastatic poorly differentiated gastric carcinoma showing positive cytoplasmic stain for CK 18 (ABC, X400). B) Metastatic poorly differentiated biliary tract carcinoma, showing positive staining to CA19-9 (ABC, X200).**

## Discussion

The liver is a very common target of metastatic tumors. About 40% of patients who die of cancer have liver metastasis and 10% die of hepatic failure<sup>[5,6]</sup>. According to autopsy studies, hepatic metastases most commonly originate from primary tumors of the colon, pancreas and breast<sup>[7,8]</sup>. However, the localization of the primary tumor at the time of initial clinical presentation of the metastatic disease is frequently unknown. Occult primary tumors account for 5-10% of all neoplasms<sup>[9-11]</sup>, the majority of them being adenocarcinoma<sup>[11,12]</sup>. Metastatic tumors with unknown primary site tend to have an unfavorable prognosis, but proper identification of the site of origin has prognostic and therapeutic significance<sup>[13-15]</sup>. The search for an unknown primary tumor is, however, often time-consuming and unrewarding<sup>[15]</sup>.

Immunohistochemical phenotyping of primary and metastatic tumors in the liver gave promising results and helpful in the clinical search for the primary tumors, but the diverse and complex algorithms proposed could not be widely accepted in clinical practice<sup>[16,17]</sup>. More



recently, attention has been focused on CK expression in adenocarcinoma and other tumors, especially on the expression of CK20 and CK7. The combination of these two monoclonal antibodies has been found to be helpful in discriminating primary and metastatic tumors in the ovaries, lungs, pleura and other organs. Results of CK20/CK7 phenotyping of metastatic carcinomas of unknown primary site have been also reported<sup>[18-24]</sup>. The expression of CKs in many different tumor types has become the subject of study in diagnostic pathology, yielding a large body of somewhat conflicting information, reviewed by Wang *et al.*<sup>[25]</sup>, Chu *et al.*<sup>[26]</sup> and Tot<sup>[27]</sup>.

In the present study, CK 18 was demonstrated in 87.5% of hepatocellular carcinomas, all cholangiocarcinoma, and all metastatic carcinomas.

On the other hand, a study by Stroescu *et al.*<sup>[28]</sup> showed that for CK 18, 70% of HCCs and only 20% of cholangiocarcinoma were diffusely positive. Positivity of CK18 in most of the tested carcinomas was explained by Moll<sup>[29]</sup>, who stated that CK 18 is a low molecular CK that can be expressed in a simple, non-stratified epithelium. In addition, superficial layer of transitional epithelium and secretory cells of complex epithelium makes CK18 of no benefit in the differential diagnosis of primary hepatic carcinoma, and metastatic cases from any site.

In the present study, 87.5% of HCC cases showed CK7-ve/CK20-ve pattern, whereas, 12.5% of cases were CK7+ve/CK20-ve. These findings are in concordance with previous study, which showed that approximately 15% (range 0-25%) of HCCs stain positively to CK7. These cases constitute either biliary differentiation in otherwise typical HCC or they are mixed HCC-cholangiocarcinoma cases. Other authors do not view focal staining with biliary type's keratin alone, as sufficient criteria to label an otherwise typical HCC as mixed HCC-CC. They restrict the use of mixed HCC-CC to well differentiated tumors that display obvious CC adjacent to HCC<sup>[30,31]</sup>. Sell and Dunsford proposed from studying experimental hepatic carcinogenesis that liver cell and bile duct cell arise from a pluripotent stem cell and there is some evidence of such common origin in humans. This common origin enables the benign hepatocytes to transform into bile ductules [under certain pathologic conditions]<sup>[30]</sup>.

Cholangiocarcinoma, gastric carcinoma and pancreaticobiliary carcinoma cases were CK7+ve/CK20-ve/CA19-9+ve, whereas, metastatic carcinoma from the colorectal region were CK7-ve/CK20+ve/CA19-9+ve. These features were also noted by Stroescu *et al.* that showed that all cholangiocarcinoma were diffusely positive to CK 7<sup>[28]</sup>.

In similar studies, Tot<sup>[19,21]</sup> found that metastatic adenocarcinoma from the stomach showed a variable immunostaining pattern; 50% were stained positively for CK20, 60% were stained positively for CK7, and 40% were negative. He also stated that 87% of metastases from the pancreas were CK7+, but there was a considerable variation in the CK20 staining of these metastases, where 22% were diffusely positive, and 17% were focally positive. The CK20+/7- phenotype indicates metastatic adenocarcinoma, most often from the colon or rectum. Tot<sup>[19]</sup> stated that CK20 antibody labels the majority of adenocarcinoma of the colon, mucinous ovarian tumors, and transitional. Merkel cell carcinomas are often positive in adenocarcinoma of the stomach, bile duct, gallbladder, and pancreas.

Although AFP is the most useful serum tumor marker for the diagnosis of liver cell carcinoma, the incidence of AFP-positive HCC has varied among studies, and it appears not always to be helpful in the histological diagnosis of all cases of HCC. Many studies showed that the presence of AFP was not specific for HCC. As serum AFP levels are elevated in as many as 20% of patients with cholangiocarcinoma, and even in other types of carcinomas, including gastric, pancreatic, colonic and ovarian carcinomas<sup>[3,4,12,13]</sup>. In the present study, about 44% of HCC showed positive AFP immunostaining, while it was negative in cholangiocarcinoma and metastatic carcinomas from colorectum, stomach and pancreaticobiliary region. A wide variation in the incidence of AFP positive HCC has been reported to date. Hurlimann and Gardiol<sup>[1]</sup> obtained staining for AFP in 61.5% of HCC cases. Brown *et al.*<sup>[16]</sup> demonstrated positive immunoreactivity to AFP in 16/63 (24%) HCC. He found that positivity is directly related to tumor grade and high serum level. In most studies, the incidence of AFP positive HCC was within the range of 12–50%<sup>[12-16]</sup>. The variation in the incidence is probably attributed to the marked variation of positivity to the weak staining. In many instances, differences in the specificity or affinity of anti-AFP



antibodies, or due to the differences in the fixation of materials, and in the immunohistochemical methods<sup>[17]</sup>.

The carbohydrate antigen was used for a long time as a serum marker for the diagnosis of pancreatic and colonic carcinomas<sup>[32]</sup>. In the present study CA19-9 was positive in all colonic, pancreaticobiliary, and most gastric carcinomas; also it was positive in cholangiocarcinoma cases. Similar to CK7, positivity to Ca19-9 can discriminate between cases of primary hepatocellular carcinoma from CC and metastatic carcinoma, but cannot differentiate between CC and metastatic carcinoma.

### Conclusion

CK18 is of no benefit in the differentiation between primary hepatic carcinoma and metastatic cases from gastrointestinal tract. CK7 and CA19-9 positive staining can exclude a diagnosis of hepatocellular carcinoma, but cannot discriminate between metastatic carcinoma (from stomach and pancreaticobiliary origin) and cholangiocarcinoma. The CK20+/CK7-ve phenotype indicates metastatic intestinal adenocarcinoma, most often from the colon or rectum.

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## الفائدة التشخيصية للصبغات المناعية في التفريق بين الأورام الأولية والثانوية في الكبد

علي صادق صوان

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المستخلص. أورام الكبد السرطانية الثانوية والغير معروف مصدرها الأولي تعتبر مشكلة إكلينيكية. ويطلب من أطباء علم الأمراض عادة التفريق بين الأورام السرطانية الأولية في الكبد والأورام السرطانية الثانوية. ولأن مكونات تلك السرطانات تتشابه لحد كبير في الاختبار النسيجي المجهرى فإن استخدام الصبغات المناعية قد يكون له فائدة كبيرة في التفريق بين تلك الأورام. ولكون بعض الصبغات المناعية التي استخدمت سابقاً في ذلك الغرض لم تعد بفائدة كبيرة فقد تم اختبار مجموعة أخرى من تلك الصبغات المناعية لإجراء الدراسة عليها.