Differential expression of MOC-31, Hep Par 1, and N-cadherin in primary carcinoma and metastatic adenocarcinoma in the liver

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Background
Immunohistochemistry plays a crucial role in the diagnosis of hepatocellular carcinoma (HCC) and in its distinction from other primary and metastatic neoplasms. In this study, we examined the expression of MOC-31 (Anti-epithelial cell adhesion molecule monoclonal antibody, clone number-31), hepatocyte paraffin 1 (Hep Par 1), and N-cadherin in primary carcinoma and metastatic adenocarcinoma (AC) in the liver.

Aim
The aim of this study was to evaluate the usefulness of MOC-31, Hep Par 1, and N-cadherin in the differential diagnosis of primary carcinoma and metastatic AC in the liver.

Materials and methods
The present study included 56 specimens from cases of primary and metastatic liver tumors, including 20 primary HCCs in the liver, five intrahepatic cholangiocarcinomas, and 31 metastatic ACs in the liver. They were studied to evaluate MOC-31, Hep Par 1, and N-cadherin expression using immunohistochemistry.

Results
The sensitivity of MOC-31 for AC in the studied group was 97.2%, whereas its specificity was 90%. The sensitivity of Hep Par 1 for HCC was 75%, whereas its specificity was 100%. The sensitivity of N-cadherin for primary liver carcinoma was 72%, whereas its specificity was 83.9%. Using the combination of the three antibodies, a final diagnosis could be established in 52 of 56 (92.9%) cases of studied group. In conclusion, a panel of these three antibodies can be helpful in the distinction between primary carcinoma and metastatic AC in the liver.

Keywords:
cholangiocarcinoma, hepatocyte paraffin 1, hepatocellular carcinoma, immunohistochemistry, metastatic adenocarcinoma, MOC-31, N-cadherin

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Introduction

Liver cancer is the sixth most common cancer worldwide; its very poor prognosis makes it the third leading cause of cancer-related mortality, responsible for about 600 000 deaths annually [1]. In Egypt, it is reported that the age-specific incidence rates of liver cancer are 61.8/100 000 for male and 24.4/100 000 for female population. Considering both sexes, liver carcinoma is the most common cancer in Egypt, accounting for about 23.81% of all cancers [2].

In upper Egypt, the incidence rates for liver carcinoma are much lower than those in other areas of the country. This can be attributed to the fact that liver cancer in Egypt followed the distribution of hepatitis C virus infection, which is more frequent in Nile delta, with decreasing prevalence going south [3].

Hepatocellular carcinoma (HCC) represents 70–85% of primary liver cancers; cholangiocarcinoma (CC), which originates from cholangiocytes, constitutes 10–15% of primary hepatic malignancies. The remaining 5% are uncommon tumors such as primary liver angiosarcoma, hepatic epithelioid hemangioendothelioma, hemangiopericytoma, or primary hepatic lymphoma [4].

The liver is a very common target of metastatic tumors. According to autopsy studies, hepatic metastases most commonly originate from primary tumors of the colon, pancreas, and breast. 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, the majority of them being adenocarcinoma (AC) [5].

The distinction of HCC from CC and other types of AC metastatic to the liver is a relatively frequent, often challenging, dilemma for surgical pathologists and very crucial, as the treatment goals for these tumors are different. Although, in most cases, the correct

diagnosis can be reached through a synthesis of clinical findings, diagnostic imaging modalities, and routine evaluation of hematoxylin and eosin-stained sections, immunohistochemistry (IHC) may play a very valuable role in clinically atypical and pathologically indeterminate cases. It is challenging because limited tissue is available with core biopsies, and hence an appropriate selection of antibodies is imperative [6].

MOC-31 is a monoclonal antibody that recognizes the extracellular domain EpEX of epithelial cell adhesion molecule, which is a type I transmembrane glycoprotein. It is expressed on the basolateral membrane in most normal epithelial tissues and is overexpressed in many human carcinomas [7].

MOC-31 has been reported to be a useful marker in the IHC panel used to distinguish AC from malignant mesothelioma in many studies [8,9]. In the liver, MOC-31 is expressed in more than 90% of CC and metastatic AC (including colorectum, pancreas, stomach, lung, breast, and ovary). The majority of HCCs are negative or weakly positive [10].

Hepatocyte paraffin 1 (Hep Par 1) is an antibody for carbamoyl phosphate synthetase 1, a urea cycle enzyme in hepatocellular mitochondria, which is expressed predominantly in the liver [11]. Wennerberg *et al.* [12] reported the development of this monoclonal antibody and designated it as Hep Par 1; it was produced in mice using tissue from a failed allograft liver.

This antibody has been found to be relatively sensitive and specific for hepatocellular differentiation in normal tissue and HCC, as well as hepatoblastoma [6]. However, through many years of use, many of the pitfalls of Hep Par 1 have been elucidated. For example, it marks hepatoid tumors of any organ [13].

Cadherins are single transmembrane proteins that form especially with catenins, a calcium-dependent cell–cell adhesion complex called adherent junction [14]. N-cadherin is a member of the type I classical cadherin subfamily. Depending on the cell type, the expression of N-cadherin can lead to different cellular behavior through the activation of different signaling pathways [15].

In the gastrointestinal tract, N-cadherin expression is liver specific because both hepatocytes and intrahepatic biliary epithelial cells strongly express this marker at their plasma membrane, and hence its expression strongly argues for the primary origin of a liver tumor. An interesting point is that N-cadherin is not expressed by extrahepatic bile ducts. This can be attributed to the different embryological origins [16].

Aim

The aim of this study was to study IHC expression of MOC-31, Hep Par 1, and N-cadherin in primary carcinoma and metastatic AC in the liver, and to evaluate the usefulness of this IHC panel in differentiating primary carcinoma from metastatic AC in the liver.

Materials and methods

The present study included randomly chosen 56 specimens of primary liver carcinomas and metastatic ACs in the liver. Twenty of them were diagnosed as primary HCC in the liver, five were diagnosed as intrahepatic CC, and 31 specimens were metastatic ACs. The pancreas followed by the colon and then the stomach were the most prevalent primary sites of metastatic AC in the studied group.

Tumor classification was performed according to WHO criteria [17], and HCC cases were graded as grades 1, 2, and 3 according to the classification of Jain [18].

The paraffin-embedded blocks for each specimen were dissected and subjected to the following:

(1) Routine hematoxylin and eosin staining to confirm the original diagnosis
(2) IHC staining of Hep Par 1, MOC-31, and N-cadherin antibodies utilizing the avidin–biotin–immunoperoxidase complex technique.

The avidin–biotin–peroxidase complex IHC method was performed on sections placed on positively charged slides. The slides were deparaffinized in xylene, and rehydrated in graded alcohols. They were then incubated with hydrogen peroxide block and then rinsed in PBS, pH 7.4. Subsequently, incubation with a primary antibody was performed.

The primary antibodies used in the study to stain the tumor sections were MOC-31, Hep Par 1, and N-cadherin.

MOC-31

Incubation with primary MOC-31 mouse monoclonal antibody was carried out for 45 min at room temperature (clone MOC-31, 1 : 200 dilution; Biocare Medical, Concord, CA 94520 USA).

Hep Par 1

Incubation with primary Hep Par 1 mouse monoclonal antibody was carried out for 30 min at room temperature (clone OCH1E5, 1 : 40 dilution; Thermo Scientific).

N-cadherin

Incubation with primary N-cadherin mouse monoclonal antibody was carried out for 30 min at room temperature (clone 13A9, 1 : 100 dilution; Novus Biologicals).

Antigen detection was carried out through exposure to a biotinylated universal secondary antibody, followed by exposure to a streptavidin–peroxidase complex working solution. The antigen–antibody complex was visualized by staining with diaminobenzidine/hydrogen peroxidase chromogen solution. The sections were counterstained with Mayer's haematoxylin, dehydrated in graded alcohols followed by xylene, and then mounted in a DPX mounting medium.

Scoring system

MOC-31 was expressed in a membranous pattern and the tumor was considered positive for this antibody if more than 5% of the tumor cells showed membranous staining. This cutoff value was selected from the study by Karabork *et al.* [19]. Positive reaction for Hep Par 1 was defined as diffuse cytoplasmic staining with moderate-to-strong intensity involving greater than 10% of tumor cells. This cutoff value was selected from the study by Shiran *et al.* [20]. N-cadherin labeling was scored as positive if more than 10% of the tumor cells showed membranous staining. This cutoff value was selected from the study by Hooper *et al.* [21].

Statistical analysis

The data were collected, tabulated, and statistically analyzed using the statistical package for the social sciences (SPSS, version 16; SPSS, Chicago, Illinois, USA) for windows. Rates and proportions were calculated for categorical data, and the x^2 and Fisher's exact tests were used to analyze the statistical differences between qualitative categorical variables. The diagnostic value of each immunoprofile was analyzed according to its sensitivity and specificity.

Results

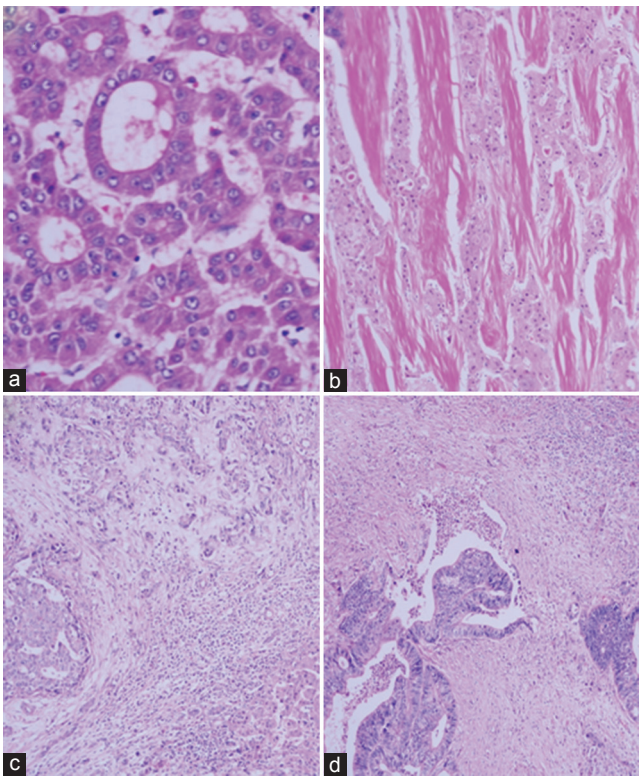
Of the 56 specimens in this study, 20 were HCC, five were CC, and 31 specimens were metastatic ACs (Fig. 1). The primary site of metastatic AC was the pancreas in seven cases, the colon in five cases, the stomach in four cases, the uterus in one case, and the breast in one case. Thirteen of 31 metastatic AC cases were of unknown primary origin.

Results of IHC staining of the specimens of the studied group with the three antibodies are summarized in Table 1. The sensitivity and specificity of the three antibodies to the different types of tumors included in the study are shown in Table 2.

As regards MOC-31, 30 of 31 (96.8%) metastatic AC cases and all five (100%) CC cases were positive, whereas only two of 20 (10%) HCC cases were positive for this antibody immunostaining (Fig. 2). The sensitivity of MOC-31 for AC in the studied group was 97.2%, whereas its specificity was 90%.

As regards Hep Par 1, 15 of 20 (75%) HCC cases were positive, whereas none of the CC or metastatic AC cases was positive for this antibody (Fig. 3). The

Figure 1



(a) Hepatocellular carcinoma, pseudoglandular pattern. (b) Hepatocellular carcinoma, fibrolamellar variant. (c) Intrahepatic cholangiocarcinoma. (d) Metastatic colonic adenocarcinoma in the liver. Hematoxylin and eosin, (a) ×400; (b–d) ×200.

Table 1 Results of immunohistochemistry of all tumors included in the study

Cases (*n*=56)	MOC-31 (*N* (%))	Hep Par 1 (*N* (%))	N-cadherin (*N* (%))
HCC (*n*=20)	2/20 (10)	15/20 (75)	14/20 (70)
CC (*n*=5)	5/5 (100)	0/5 (0)	4/5 (80)
Metastatic AC (*n*=31)	30/31 (96.8)	0/31 (0)	5/31 (16.1)
Pancreas	7/7	0/7	1/7
Colon	5/5	0/5	0/5
Stomach	4/4	0/4	0/4
Uterus	1/1	0/1	0/1
Breast	1/1	0/1	0/1
Unknown	12/13	0/13	4/13

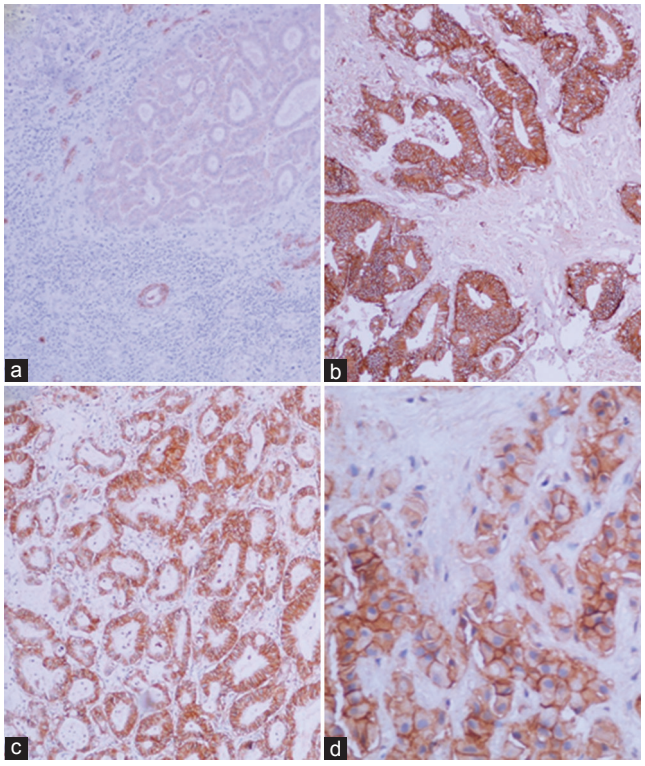
AC, adenocarcinoma; CC, cholangiocarcinoma;
HCC, hepatocellular carcinoma; Hep Par 1, hepatocyte paraffin 1.

Table 2 Sensitivity and specificity of the three antibodies in diagnosing cases of the study group

	HCC (*n*=20) (*N* (%))	CC (*n*=5) (*N* (%))	Metastatic AC (*n*=31) (*N* (%))	SN	SP	*P*
MOC-31	2 (10)	5 (100)	30 (96.8)	97.2	90	<0.0001**
Hep Par 1	15 (75)	0 (0)	0 (0)	75	100	<0.0001**
N-cadherin	14 (70)	4 (80)	5 (16.1)	72	83.9	<0.0001**

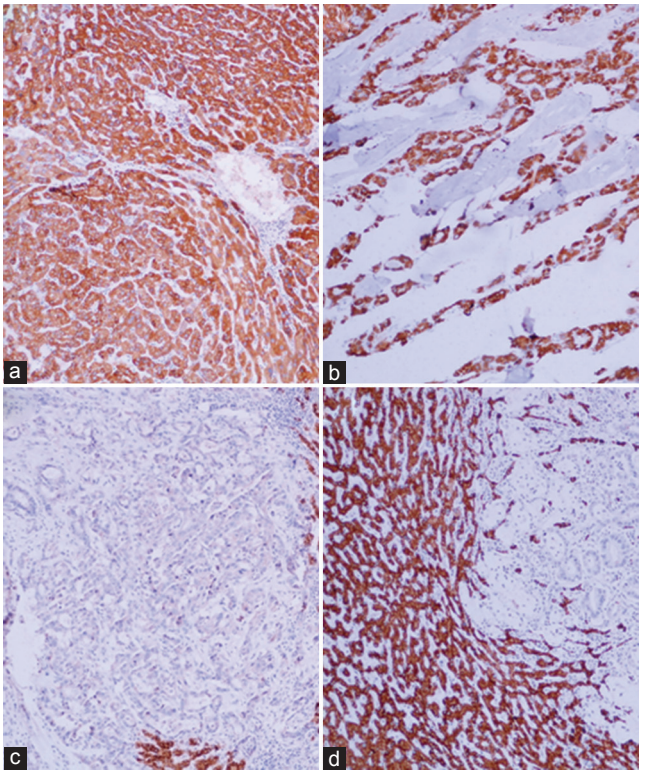
AC, adenocarcinoma; CC, cholangiocarcinoma; HCC, hepatocellular carcinoma; Hep Par 1, hepatocyte paraffin 1; SN, sensitivity; SP, specificity. P-value using Fisher's exact test. **Highly significant.

Figure 2



MOC-31 immunohistochemical expression: (a) hepatocellular carcinoma (pseudoglandular pattern) negative for this antibody with positive non-neoplastic bile ducts and ductules; (b) metastatic colonic adenocarcinoma showing membranous reactivity; (c) metastatic gastric adenocarcinoma showing membranous reactivity; (d) metastatic breast carcinoma showing membranous staining for this antibody. Diaminobenzidine chromogen, hematoxylin counterstain, (a–c) ×200; (d) ×400.

Figure 3



Hepatocyte paraffin 1 immunohistochemical expression: (a) conventional hepatocellular carcinoma showing diffuse cytoplasmic staining; (b) fibrolamellar variant with positive cytoplasmic staining; (c) cholangiocarcinoma negative to this antibody with positive adjacent non-neoplastic hepatocytes; (d) metastatic gastric adenocarcinoma negative to this antibody with positive adjacent non-neoplastic hepatocytes. Diaminobenzidine chromogen, hematoxylin counterstain, (a–d) ×200.

sensitivity of Hep Par 1 for HCC in the studied group was 75%, whereas its specificity was 100%.

As regards N-cadherin IHC staining, 14 of 20 (70%) HCC cases, four of five (80%) CC cases, and five of 31 (16.1%) metastatic AC cases were positive for this antibody (Fig. 4). The sensitivity of N-cadherin for primary liver carcinoma in the studied group was 72%, whereas its specificity was 83.9%.

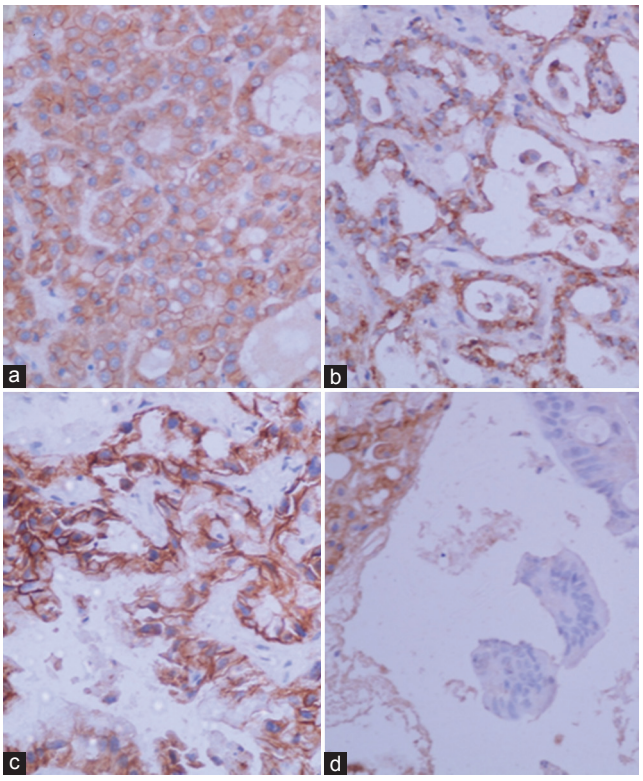
The combination of the three antibodies was helpful in diagnosing 52/56 (92.9%) cases. Only four cases remained equivocal using this combination. For a diagnosis to be considered definitive, at least one of the antibodies had to be positive (Hep Par 1 and N-cadherin for HCC; MOC-31 and N-cadherin for CC; and MOC-31 for AC). Cases were considered equivocal when no positive staining was obtained with any of the antibodies considered.

Table 3 demonstrates the differential expression of MOC-31, Hep Par 1, and N-cadherin combination in HCC, CC, and metastatic AC. Cases that were positive for MOC-31 and negative for both Hep Par 1 and N-cadherin were more likely to be metastatic AC with a high statistical significance, whereas cases that were negative for MOC-31 and positive for both Hep Par 1 and N-cadherin were more likely to be HCC with a high statistical significance. Cases that were positive for both MOC-31 and N-cadherin and negative for Hep Par 1 were more likely to be CC with a statistical significance.

Discussion

As regards differentiation between primary liver carcinoma and metastatic AC to the liver, clinical information on serum tumor marker levels and radiological findings are helpful. A tissue diagnosis is necessary to make a definitive diagnosis. The pathologist uses morphology to establish a differential diagnosis and then uses histochemical and IHC studies to refine the diagnosis. IHC is helpful when morphology and identification of secretory substances fail [22]. In this study, we tried to examine the usefulness of MOC-31, Hep Par 1, and N-cadherin in the differential diagnosis of HCC, CC, and metastatic AC in the liver.

Figure 4



N-cadherin immunohistochemical expression: (a) hepatocellular carcinoma with pseudoglandular pattern showing membranous staining for N-cadherin; (b) cholangiocarcinoma showing membranous staining for this antibody; (c) metastatic pancreatic adenocarcinoma with membranous reactivity to this antibody; (d) metastatic colonic adenocarcinoma negative for N-cadherin with membranous staining of non-neoplastic hepatocytes. Diaminobenzidine chromogen, hematoxylin counterstain, (a–d) ×400.

In the present study, 30 of 31 metastatic AC cases and all five CC cases showed positive membranous immunoreactivity for MOC-31, with 97.2% sensitivity to AC in the studied group. Only two of the 20 HCC cases showed membranous positivity for MOC-31, and hence the specificity of MOC-31 was 90%. A fairly similar finding was observed by Wang *et al.* [23], who observed that 97% of metastatic ACs and 6% of HCCs in their study were positive for MOC-31.

Proca *et al.* [24] reported no MOC-31 staining in HCCs. This finding was confirmed by Porcell *et al.* [25]. In contrast to these results, Lau *et al.* (2002) noted MOC-31 expression in five of 42 (12%) HCCs. Morrison *et al.* [26] found that one of 25 (4%) HCCs was positive with MOC-31. Our findings confirm the previous results with MOC-31 in HCCs. We found a similar trend in favor of MOC-31 negativity in HCCs and MOC-31 positivity in metastatic ACs, suggesting that MOC-31 is a valuable marker in the differential diagnosis.

An obviously lower sensitivity (65%) of MOC-31 was observed by Al-Muhannadi *et al.*[27]. However, they had used a more concentrated antibody (1 : 50, dilution), and, in contrast to the vast majority of studies in the literature, including ours, they considered cases to be positive when the staining had been strong and diffuse with a cytoplasmic pattern. They did not give any reason for this contravention in interpretation of positivity.

As regards Hep Par 1 expression, we found positive immunostaining for this antibody in 15 of the 20 HCC cases, and hence the sensitivity of this antibody for HCC in the studied group was 75%. Our results are in agreement with most other studies in the literature, in which the sensitivity of this antibody for HCC ranged from 66% [28] to 96.6% [29].

Table 3 Differential expression of MOC-31, hepatocyte paraffin 1, and N-cadherin combination in hepatocellular carcinoma, cholangiocarcinoma, and metastatic adenocarcinoma

MOC-31	Hep Par 1	N-cadherin	HCC (*N* (%))	CC (N (%))	Metastatic AC (*N* (%))	*P*
+	+	+	1/20 (5)	0/5 (0)	0/31 (0)	0.4
+	+	–	0/20 (0)	0/5 (0)	0/31 (0)	NA
+	–	+	1/20 (5)	4/5 (80)	5/31 (16.2)	0.0004*
+	–	–	0/20 (0)	1/5 (20)	25/31 (80.6)	<0.0001**
–	–	–	2/20 (10)	0/5 (0)	1/31 (3.2)	0.49
–	+	+	10/20 (50)	0/5 (0)	0/31 (0)	<0.0001**
–	+	–	4/20 (20)	0/5 (0)	0/31 (0)	0.02*
–	–	+	2/20 (10)	0/5 (0)	0/31 (0)	0.15

AC, adenocarcinoma; CC, cholangiocarcinoma; HCC, hepatocellular carcinoma; Hep Par 1, hepatocyte paraffin 1; NA, not applicable. *P*-value using the χ^2-test. *Significant. **Highly significant.

The specificity of Hep Par 1 in this study was 100%; all non-HCC cases were negative for this antibody. This is in agreement with the results of most studies in this issue, in which the specificity of Hep Par 1 ranged from 87.7% (Lau *et al.*, 2002) to 100% [26]. An apparently lower specificity (63.6%) was observed by Lee *et al.* [30]; however, they used a lower cutoff value (5%).

In the present study, N-cadherin showed the expected membranous staining pattern and stained 14 of 20 (70%) HCC cases and four of five (80%) CC cases. Only five (16.2%) of 31 metastatic AC cases showed positivity for this antibody. The sensitivity of N-cadherin for primary liver carcinoma in the present study was 72% and its specificity was 83.9%. We considered tumor cells to be positive for N-cadherin when they had shown membranous and/or combined membranous and cytoplasmic staining. We labeled cases that showed only cytoplasmic staining as negative for this antibody. This is in agreement with the positivity evaluation method in the study by Cho *et al.* [31].

As regards N-cadherin expression in HCC, our results are in concordance with the study by Kozyraki *et al.* [32], who reported N-cadherin expression in 36/95 (55.4%) of their HCC cases. Other higher percentages were reported by other authors such as Tajima *et al.* [33], who reported N-cadherin expression in 20 of 26 (76.9%) HCCs, and Cho *et al.* [31], who reported N-cadherin expression in 64 of 68 (94%) HCC cases.

Mosnier *et al.* [16] reported N-cadherin membranous immunoreactivity in all 22 (100%) HCC and in 23 of 29 (79%) intrahepatic CC cases, whereas none of the 32 (0%) metastatic ACs to the liver was positive for N-cadherin in their study.

In their study to distinguish pancreatic ductal AC from CC, Hooper *et al.* [21] found N-cadherin membranous expression in five of 23 (22%) metastatic pancreatic AC to the liver, whereas 17 of 27 (63%) intrahepatic CC were positive for this antibody. Only one of four (25%) extrahepatic CC cases was positive for N-cadherin, whereas none of the 14 metastatic AC cases (from the gall bladder, ampulla, and colon) was positive for this antibody.

In apparent contrast with our study, an abnormal N-cadherin expression was reported by Nakajima *et al.* [34], who reported that eight of 15 (53%) metastatic pancreatic ACs to the liver were positive for N-cadherin expression. However, this expression was localized within the cytoplasm of the tumor cells, sparing their plasma membranes, and they considered tumor cells that had shown only cytoplasmic staining for N-cadherin to be positive for this antibody.

A combination of MOC-31 and Hep Par 1 was the most useful combination of two antibodies in our study as it distinguished HCC from AC in 50/56 (89.3%) of our cases. This finding is in concordance with the results of other studies such as Morrison *et al.* [26], in which correct diagnosis was achieved in 90 of 100 cases of HCC and AC using this combination of the two antibodies.

Addition of N-cadherin to the combination of MOC-31 and Hep Par 1 added to this combination the benefit of distinguishing CC from metastatic AC. The cases that were positive for both MOC-31 and N-cadherin and negative for Hep Par 1 were significantly more likely to be CC. To the best of our knowledge, no previous study has evaluated this panel before ours. However, it was a useful combination of three antibodies diagnosing 52 of 56 (93%) of our cases.

In conclusion, the use of MOC-31, Hep Par 1, and N-cadherin together in a panel can solve most problems in the distinction between primary carcinoma and metastatic AC in the liver.

Conclusion

An immunohistochemical panel formed of MOC-31, Hep Par 1 and N-cadherin can be helpful in the distinction between hepatocellular carcinoma, intrahepatic cholangiocarcinoma and metastatic adenocarcinoma in the liver.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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