Efficacy of Expressions of Arg-1, Hep Par-1, and CK19 In the Diagnosis of the Primary Hepatocellular Carcinoma Subtypes and Exclusion of the Metastases

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Running title

Expression of Arg-1, Hep Par-1, CK19 in diagnosis of hepatocellular carcinoma subtypes

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Abstract

Many conflicts arise using immunohistochemistry of Hepatocellular carcinoma (HCC), some of these conflicts arise from the biliary part within the tumor itself or from liver metastasis. The aim of this study is to investigate the extent of Arg-1, HepPar-1, and CK-19 expressions in the primary HCC subtypes as well as studying of some metastatic cases to find a distinctive immunohistochemical panel utilizing it to differentiate between these entities. Material and methods: A paraffin-embedded block including 62 cases of primary HCC, and 18 cases diagnosed as metastatic tumors, were subjected for this study using Anti-liver Arginase antibody (ab125134 Cambridge, USA, polyclonal antibody, 3.75 µg/ml), HepPar-1 (polyclonal mouse antibody OCH1E5; 1:600; DAKO, CA, USA), and CK 19 Anti-Cytokeratin 19 antibody (ab15463, rabbit polyclonal antibody; 1:100; Cambridge, USA). The intensity of immunostaining was scored (0 to 3+). Nuclear and cytoplasmic staining with Arg-1 and cytoplasmic for both HepPar-1 and CK 19 are reported. Results: The histopathological patterns were mainly trabecular no= (24, 38.7%), and pseudoglandular (no=14, 22.5 %), mixed hepatocellular cholangiocarcinoma was observed in one case (1.6%). Arginase-1 positivity was in 55 cases (88.7%) opposite to 46 (74.19%) and 8 (12.9%) for HepPar.1% -1 and CK 19, respectively. The intensity of expression was marked in well and moderate differentiation for Arg-1 and HepPar-1and in poorly differentiated for CK 19. Metastatic carcinoma cases revealed two cases positive for Arg-1 (11.1%), 4 cases (22.2%) positive for HepPar-1, and 13 cases (72.2%) positive for CK 19. Conclusion: Arg-1 and HepPar-1 are confirmative in the diagnosis of primary HCC in most cases, either separately or collectively but the priority of
selection leans more towards Arg-1. Arg-1 and HepPar-1 positive with negative CK 19 expressions give more support to diagnosis of primary HCC while the reverse will support the diagnosis of tumour of biliary origin or liver metastasis.

**Keywords:** Arginase-1, CK 19, Cholangiocarcinoma, Hepatocellular carcinoma, HepPar-1, liver metastasis
Efficacy of Expressions of Arg-1, Hep Par-1, and CK19 In the Diagnosis of the Primary Hepatocellular Carcinoma Subtypes and Exclusion of the Metastases.

Introduction

Hepatocellular carcinoma (HCC) is one of the most widespread malignancies of the liver representing 70–85 % of overall liver cancers around the world (Jemal et al., 2011). Hepatocellular carcinoma (HCC) can be subclassified into various histologic sub-categories depending on the architecture and cytologic features. Three distinctive architectural patterns are observed in “classical” HCC and frequently co-exist in one tumour. The most widespread is the trabecular a pattern that is characterized by tumour cells assembled in thick cords encircled by endothelial cells creating sinusoidal-like spaces. The second pattern is acinar or pseudoglandular and reveals tumour cells formed into gland-like structures that represent the abnormally dilated bile canaliculi. The third variant is the solid or compact pattern which is composed of sheets of hepatocytes with delicate sinusoid-like spaces, often seen in the poorly differentiated tumours. Other patterns include clear cell, giant cell, pelioid or vascular lake, sarcomatoid, and steatohepatitic HCC (Salomao et al., 2010). Other variants include combined hepatocellular cholangiocarcinoma (Willekens et al., 2009), and diffuse cirrhosis-like HCC (Jakate et al., 2010). According to the differentiation of HCCs, they can be placed as well, moderately, and poorly differentiated, depending on their similarity to the normal hepatic tissue (Salomao et al., 2012).

Arginase-1 (Arg-1) is an enzyme implicated in the urea cycle and has recently been recognized as a helpful diagnostic marker in the differential diagnosis of primary HCC from metastatic tumors (Yan et al., 2010; Timek et al., 2012). However, because of its involvement in the
hydrolysis of arginine to ornithine and urea, it was found that Arg-1 expression was observed in tumors related to hepatocytic origin whether benign or malignant, including hepatic adenoma and primary HCC, hence it is less valuable in differentiating between them (Sang et al., 2015). Even in HCC, the previous studies were inconsistent between studies. Some of the main reasons for the disagreements among the previous studies were the small sample size and low number of cases reported in these studies (Fujiwara et al., 2012; McKnight et al., 2012). So, a more accurate diagnosis cannot be achieved via insufficient samples especially in heterogenous tumors.

Hepatocyte paraffin-1 (HepPar1) is a reliable hepatocyte lineage marker and is also regarded as a potent tool in the differential diagnosis of hepatocellular tumors (Al-Muhannadi et al., 2011; Shibuya et al., 2011). The antigen for HepPar1 was reported to be the hepatocytic urea cycle enzyme, carbamoyl phosphate synthetase 1 located in mitochondria (Butler et al., 2008).

Numerous sensitivities were noticed for HepPar-1 in the diagnosis of primary HCC, but these sensitivities were found to be related to the degree of differentiation. In well-differentiated tumors, the sensitivity was around 80%, while in primary poorly differentiated HCC it was around 50 %, despite being progressively used as a positive marker for confirmation of its hepatic origin (Lau et al., 2002; Kakar et al., 2003; Wang et al., 2006; Nguyen et al., 2015). Recently, growing evidence has supported the presence of strong HepPar-1 expression in some tumors rather than HCC, as adenocarcinoma of the oesophagus, gastric, and lung (Chu et al., 2002; Fan et al., 2003; Kakar et al., 2007). In addition, HepPar-1 expression was found focally positive in several tumors as melanoma, adrenocortical carcinomas, renal cell
carcinoma, neuroendocrine tumor, and epitheloid angiomyolipoma (Krings et al., 2013; Fujikura et al., 2016).

Cytokeratins (CK) are intermediate cytoskeletal filaments present in both normal and carcinoma cells (Uenishi et al., 2003). In normal hepatocytes, CK8 and CK18, are expressed while bile duct cells express both CK7 and CK19 (Durnez et al., 2006). This distribution of CK has been thought to be preserved during tumorigenesis, thus primary HCC would be anticipated to express either CK8 or CK18 or both but not express CK7 or CK19 (Park et al., 2007; Park, 2011).

**Aim of the work.**

The aim is to investigate the extent of Arg-1, HepPar-1, and CK-19 expressions in the primary HCC subtypes as well as studying some metastatic cases in an attempt to find a distinctive immunohistochemical panel, utilizing it later on to differentiate between the primary hepatocytic tumors with other lesions and reach an accurate diagnosis, especially in conflicting cases.

**Materials and methods**

This study is retrospective, and ethical approval was taken from the ethical committee of Al-Azhar university hospitals under Lab/Path; 015/2019/12/07 and comprises the utilization of archival paraffin blocks in conducting the current research.

Eighty-five tissue specimens in the form of formalin-fixed, paraffin-embedded blocks including 65 cases of primary HCC, and 20 cases diagnosed as metastatic tumors, were
collected from archives of Al-Azhar university hospitals during 2016–2019. All blocks were cut into 4µm sections and stained for hematoxylin and eosin and re-examined histologically to confirm the diagnosis. The liver secondaries originated from infiltrating ductal carcinoma of breast (n=4), lung adenocarcinoma (n=6), colonic adenocarcinoma (n=6), pancreatic carcinoma (n=2).

**Immunohistochemistry workup**

The main antibodies included in the current study: Arginase-1; Anti-liver Arginase antibody (ab125134 Cambridge, Massachusetts, USA) (polyclonal antibody using a concentration of 3.75 µg/ml), HepPar-1 (polyclonal mouse antibody OCH1E5; 1:600; cytoplasm; DAKO, Carpinteria, CA), and CK 19 Anti-Cytokeratin 19 antibody (ab15463) (rabbit polyclonal antibody; 1:100; cytoplasm; Cambridge, Massachusetts, USA). 5-µm serial sections were collected onto poly-L-lysine-coated slides and treated with a basic streptavidin peroxidase procedure utilizing a biotin-free detection system (Dakao, Colorado, USA) following a heat-induced antigen retrieval technique.

Tissue sections were first deparaffinized and hydrated in xylene and serial descending concentrations of alcohol followed by rinsing in PBS for 5-10 minutes. The second step was to do the antigen retrieval that was performed by treating the tissue sections with citrate buffer of pH 6.0 for 10 min in a 700-W microwave oven and cooled for 10 minutes before the immunohistochemical staining procedure. The third step was to block the action of endogenous peroxidase which was done by incubating the slides in hydrogen peroxide of 3% concentration for 7 minutes, and then washed in the buffer solution. After blocking the peroxidase activity, the tissue was subjected for incubation with the primary antibody (Arg-1,
HepPar-1, and CK 19) for 1 h at room temperature for 30 minutes. After incubating with the primary antibodies used (Arg-1; Anti-liver HepPar-1, and CK 19), all tissues were then subjected to the rabbit anti mouse IgG (Horseradish peroxidase (HRP), Ab6728, Cambridge, Massachusetts, USA) as a secondary antibody for 1 hour at room temperature. Then the antibody reaction was detected using the avidin-biotin detection kit using diaminobenzidine (DAB) as chromogen for 5 minutes, and DAKO automation haematoxylin as counterstain for 15 minutes. The sections were washed using Tris-buffered saline. Cover slipping was then done using the automatic cover slipper; Tissue Tek SCA (Sakura Finetek USA, Torrance, CA).

Immunoreactivity scoring was semi-quantitatively done by 3 pathologists. Cases were scored as positive for all three antibodies on condition that more than 5 % of the target cells displayed the anticipated staining. The intensity of immunostaining was scored on a four-tiered scale (0 to 3+); 0? a score showing no staining, 1+ showing weak staining, 2+ showing moderate staining, and 3+ showing strong staining (Chu et al., 2002; Chu et al., 2003; Kahar et al., 2003; Timek et al., 2012; Atta, 2015, 2019). Regarding Arg-1, positive nuclear and cytoplasmic staining patterns are considered. Regarding HepPar-1 and Ck 19, a cytoplasmic staining pattern is considered. Statistical analysis of the study was done using the Kruskal-Wallis test and the two-sided p-value of ≤ 0.05 was taken into consideration. Statistical Package for social science (SPSS) version 18 has been used in this study.
Results

Histopathological examination confirmed the diagnosis of 65 cases as primary HCC and 20 cases as metastatic carcinoma. Inadequate sampling in 3 cases of primary HCC and 2 cases of metastatic carcinoma meant these cases were excluded from the current study. So, the study was carried out on 62 cases of primary HCC and 18 cases of metastatic carcinoma.

Including the 62- HCC cases, there were 38 (73.9%) male and 24 females (26.1%), the age ranged from 42–68 years with a median of 54 years. The size of the main lesion ranged from 1.6-12.2cm in its dimension with a median of 2.41 cm.

Histopathological results

The histopathological patterns were trabecular no= (24, 38.7%), pseudoglandular (no=14, 22.5%), solid (no=6, 9.67%), steatohepatitic pattern (no=5, 8%), and clear cell (no= 4, 6.4%), pelioid (vascular lake) (n=5, 8%) and sarcomatoid pattern (n=4, 6.4%).

The degree of differentiation ranged from well (no= 27, 43.5%), moderate (no= 28, 45.16%), and poorly differentiated (no= 7, 11.29%). In addition, mixed hepatocellular cholangiocarcinoma was observed in one case (1.6%). Other associated pathologic findings such as cirrhosis, necrosis and metastases outside the liver were encountered as follows: liver cirrhosis (40, 64.5%), and necrosis (22, 35.4%).
**Arg-1 immunostaining**

Arginase-1 positivity was demonstrated in 55 of 62 (88.7%) cases of HCC and the staining was strong in 23 cases (37%), moderate in 20 (32.2%), focal in 12 cases (19.3%) and negative in 11.29%. The degree of immunostaining correlated with the histologic patterns is shown in table 1,4,6 (table 1,4,7). Regarding differentiation, we found that all cases of well (27 cases, 100%), 24 out of 28 cases (85.7%) of moderate differentiation and 3 out of 7 cases of poorly differentiated (42.85%) showed positivity for Arg-1 (Fig 1-5, 9).

**HepPar-1 immunostaining**

HepPar-1 positivity was demonstrated in 46 of 62 (74.19%) cases of HCC and the staining was strong in 21 cases (33.87%), moderate in 17 cases (27.4%), focal in 8 cases (12.9%) and negative in 16 cases (25.8%). The degree of immunostaining correlated with the histologic patterns is shown in table 2, 4 and 6 (table 2,4,7).

Regarding differentiation, we found that 22 cases out of 27 well differentiated (81.48%) (Fig 1, 2), 20 cases (71.4%) of moderate differentiation (Fig 3) and 4 cases out of 7 (57.14%) of poorly differentiated showed positivity for HepPar-1 (Fig 4, 7, 9).

**CK 19 immunostaining**

CK 19 positivity was demonstrated in 8 of 62 (12.9%) cases of HCC and the staining was strong in 3 cases (4.8 %), moderate in 3 cases (4.8%), focal in 2 cases (3.2%) and negative in 54 cases (87.09%) (Fig 2,4, 8,9). The degree of immunostaining correlated with the histologic patterns is shown in table 3, 4, 6 (table 3,4,7). Regarding differentiation, we found that 2 cases out of
27 (7.4%) of well differentiated, 2 cases of moderate differentiation (7.1%) and 4 cases out of 7 (54.1%) of poorly differentiated showed positivity for CK 19.

**Results of Metastatic carcinoma**

Eighteen cases of metastatic carcinoma to liver showed the following: two cases (11.1%) showed positivity for Arg-1, 4 cases (22.2%) showed positivity for HepPar-1, and 13 cases (72.2%) revealed positivity for CK 19 (Fig 5, 9). All these metastatic carcinomas need further immunostaining evaluation. The details of immunohistochemistry results are summarized in table 4, 5, 6 and 7 (table 4-7).

Regarding the sensitivity and specificity of primary HCC to markers used in this study, the sensitivity and specificity of Arg-1 are 88.71% and 88.89% compared to 74.19% and 77.78% for HepPar-1 and 12.9% and 72.22%. for metastatic carcinoma, the sensitivity and specificity for Arg-1 was 11.11% and 11.29%, compared to 22.22% and 25.81% for HepPar-1 and 72.22% and 87.1% for CK-1.

**Discussion**

In the present study, positivity of Arg-1 was found in 55 out of 62 cases (88.7%). This is consistent with other studies such as McKnight et al. (2012), who found that Arginase-1 positivity was demonstrated in 37 of 44 (84.1%) cases of HCC, compared with 32 of 44 cases (72.7%) for HepPar-1. Also, our result is approximately near to the results of Yan et al. (2010) who found positivity of Arg-1 expression in 95.9% of the studied HCC cases.

In the present study, the results reported 7 cases (11.29%) showing negativity for Arg-1. Analysis of these cases revealed that these cases arose from 4 females and 3 males with mean
age 68 years. All these cases were associated with liver cirrhosis. Four of these cases were of moderate and 3 were of poorly differentiated HCCs and all these cases showed positivity for HepPar-1 ranging from strong (n=2), to moderate (n=5). This finding is consistent with that found in Obiorah et al. (2019) who reported 4 cases of well differentiated HCC showing negativity for Arg-1.

In the metastatic carcinoma of liver, we found two cases showing positivity for Arg-1, one from colon adenocarcinoma and another from pancreatic carcinoma. On the other hand, Fatima et al. (2014) studied Arg-1 expression in both pancreatic adenocarcinoma and primary HCC and failed to prove any significant correlation to demonstrate the hypothesis that stated stem cells of hepato-pancreatic precursor might persevere in the adult pancreas and liver and its ability to share immunophenotype. Also, this finding is consistent with that obtained by Hochstedler et al. (2013) who found positivity of Arg-1 in the lung and other tissues. However, a study of Fujiwara et al. (2012) revealed that Arg-1 is over-expressed in 9.8% of metastatic tumors.

In the present study, positivity of HepPar-1 was found in 46 out of 62 cases (74.19%). This is consistent with other studies such as McKnight et al. (2012) who found that HepPar-1 positivity was demonstrated in 32 of 44 cases (72.7%). Some authors reported that the conventionally known hepatocyte-specific markers, containing hepatocyte paraffin antigen (HepPar-1), were thought to be inadequate for the diagnosis of HCC, particularly in cases of poorly differentiated HCC (Baumhoer et al., 2008; Fujikura et al., 2016).

In the present study we found that the positivity was marked in well and moderately differentiated HCC and, in addition, the intensity of staining was more marked in trabecular, pseudoglandular and pelioid pattern. These findings are consistent with those observed by Chu et al. (2002) and Mondada et al. (2006). Chu et al. (2002) stated that the sensitivity of
HepPar-1 for HCC indicates differentiation of tumors towards hepatocytes. In addition, HepPar-1 positivity has a close relation to the grade of tumor. These suggestions are elaborated from his study which revealed that all cases with nuclear grades 1 and 2 were strongly positive for HepPar-1 compared to 84 % and 50 % for grade 3 and grade 4. Furthermore, the positive result was more marked in the trabecular and pseudoglandular pattern than of solid or compact pattern (Chu et al. 2002). In addition, steatotic, clear cells and steatohepatitic patterns showed focal staining, this coincides with Zimmermann, (2017) who found decreased reactivity in these patterns.

The results obtained showed that CK 19 positivity was seen in 8 cases (12.9%) out of 62 primary HCC and in 13 out of 18 cases (72.2%) of metastatic carcinoma. The results of primary HCC are consistent with those obtained by Witjes et al. (2013) who found that 6 out of 50 cases of non-cirrhotic liver showed positivity for CK 19 ranging from focal to moderate. Durnez et al. (2006) reported that 28% of HCCs contained cells expressing CK19 and these cells are potentially derived from hepatocytes. This is supported by identification of stem cells in HCC as well as many liver diseases (Oliva et al., 2010).

Maeda et al. (1995) advised that the primary HCC with expressive cholangiocarcinoma (CC) components should be classified as HCC-CC when the cholangiocarcinoma components are positive for biliary markers. On the other hand, some HCCs have also been reported to express CK 19 as a biliary marker even with the characteristic HCC morphologic appearance and growth pattern (Uenishi et al., 2002) although the histogenesis of these HCCs is not fully understood.

Also, our results are consistent with those obtained by Uenishi et al. (2003) who found positivity of CK 19 in 15 patients out of 157 cases with primary HCC. These findings might
indicate that CK19 expression is a predictive factor of immediate postoperative recurrence due to the increased invasiveness.

Also, our results coincide with those obtained by Zhuo et al. (2020) who found that CK19-positive HCC of positive CK19 exhibits aggressive and poor outcomes such as early recurrence and low survival rate.

In the present study, we found that 39 cases of primary HCC (62.9%) showed positivity for both Arg-1 and HepPar-1 markers which supports the diagnosis of HCC originating from hepatocytes. Out of these cases, 5 cases revealed positivity for CK19. In addition, 1 case of primary HCC showed positivity to CK19 and negativity for both Arg-1 and HepPar-1. The explanation of the five cases may be the fact that a portion of HCC is immunoreactive for cholangiocyte markers such as CK19, a fact possibly linked to the process of transdifferentiation (Shibuya et al., 2011). Furthermore, some authors found that a subtype of HCCs is distinguished by its ability to express the stemness related markers like CK19 and those tumors were designated as a dual phenotype (Kamohara et al., 2008; Kim et al., 2011; Lee et al., 2018). On the other hand, another case showed only positivity for CK 19 and this case was diagnosed as HCC of trabecular pattern and must be revised and subjected for further immunohistochemistry for its potential metastatic origin as it may be hepatoid carcinoma originating from the ovary (Wang et al., 2013) or other tissues such as ampulla of Vater, colon, bladder, kidney, pancreas, stomach, or lung.

In the present work, we found 14 cases of primary HCC showed only positivity for Arg-1. This is supported by the studies of Yan et al. (2010) and Timek et al. (2012) and Sang et al. (2015) which demonstrated that Arg-1 has a better sensitivity in identifying higher-grade HCC, including moderately differentiated and poorly differentiated HCC, than does HepPar-1. This finding is extremely useful in differentiating between a high-grade HCC from a metastasis. On
the other hand, we found five cases showed only positivity to HepPar-1, these cases must be deeply investigated to confirm its origin as cholangiocarcinoma and to exclude metastasis, especially of hepatoid carcinoma (Pitman et al., 2004).

In addition, HepPar1 reactivity was also detectable in 5 cases out of 18 (27.77%), so, despite the low number of metastatic cases, this observation coincides with many studies (Chu et al., 2003; Fan et al., 2003; Pitman et al., 2004; Wee, 2006). However, Wee, (2006) reported that not all primary HCCs stain consistently, and not every positive Hep Par1 case is of hepatocyte ancestry or occurs in the liver.

The strong point of the present study is to investigate the expression of these markers according to the histologic pattern. Although many studies deal with primary HCC few of these studies correlate their findings with the histologic patterns. Also, most liver metastases are of adenocarcinoma type but these adenocarcinoma in a number of cases are poorly differentiated and are sometimes confused with the primary HCC. In addition, some of these metastases give positive results with Arg-1 and HepPar-1. Hence, making a panel that confirms the diagnosis of primary HCC needs to be more documented. A limitation of the study is the low number of cases representing some histologic patterns of primary HCC. So, the results of correlation between immunostaining and histologic pattern must be supported by further studies with larger sample sizes of different histologic patterns. In addition, a lack of follow-up clinical data makes the correlation of the results with the prognostic factors very difficult to do.
Conclusion

Arg-1 and HepPar-1 are confirmed in the diagnosis of primary HCC in most cases, either separately or collectively, but the priority of selection is leaning more towards Arg-1. The association of the positivity for Arg-1 and HepPar-1 along with negativity of CK 19 will support the diagnosis of primary HCC while the reverse will support the liver metastases. Cases with dual biliary and hepatic origin may exhibit positivity for Arg-1, HepPar-1 and CK 19.

Conflict of interest

There is no conflict of interest to declare.

References


Figures’ legend

Fig 1. (A) Well-differentiated HCC with thick trabeculae and acini (H&E stain, x100). (B) strong staining for Arg-1 (Anti-liver Arginase antibody, x100), (C) strong staining for HepPar-1 (anti-HepPar-1 antibody, x100)

Fig 2. (A) well differentiated HCC (H&E stain, x100) (B) strong positive for Arg-1 (anti-liver Arg-1, x100) (C) strong positive for HepPar-1 (anti HepPar-1, x100) (D) Negative staining for CK 19 (anti CK-19 antibody, x100).

Fig 3. (A) Moderately differentiated HCC shows pseudoglandular pattern with moderate nuclear atypia and pleomorphism (H&E stain, x200) (B) moderate cytoplasmic staining for HepPar-1 (anti-HepPar-1 antibody, x200). This case revealed negativity for both Arg-1 and CK 19.

Fig 4. (A) Poorly differentiated HCC with thick trabeculae and acini (H&E stain, x100) (B) Strong nuclear and cytoplasmic staining for Arg-1 (Anti-liver Arginase antibody, x200), (C) Moderate membranous and cytoplasmic staining for CK 19 (anti-CK 19 antibody, x200) (D) Focal staining for HepPar-1(HepPar-1 antibody, x100).

Fig 5. (A) Metastatic carcinoma shows high nuclear atypia, pleomorphism and increased nuclear cytoplasmic ratio (H&E stain, x200) (B) moderate cytoplasmic staining for CK 19 (anti-CK 19 antibody, x200). This case revealed negativity for both Arg-1 and HepPar-1.

Fig 6. The percentage of Arg-1 reactivity in relation to histologic pattern

Fig 7. The percentage of HepPar-1 reactivity in relation to histologic pattern

Fig 8. The percentage of CK 19 reactivity in relation to histologic pattern
Fig 9. The percentage of the panel of the reactivity in both primary HCC and liver metastasis.

Table 1. The results of Arg-1 regarding the histologic pattern of the primary HCC

<table>
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<tr>
<th>Pattern</th>
<th>No.</th>
<th>%</th>
<th>Strong No.</th>
<th>%</th>
<th>Moderate No.</th>
<th>%</th>
<th>Focal No.</th>
<th>%</th>
<th>Negative No.</th>
<th>%</th>
<th>Kruskal-Wallis test</th>
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<td>11</td>
<td>45.8</td>
<td>8</td>
<td>33.3</td>
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<td>35.7</td>
<td>2</td>
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<td>20</td>
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<td>Total and % of positive cases</td>
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Table 2. The results of HepPar-1 regarding the histologic pattern of the primary HCC

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<th>Strong No.</th>
<th>%</th>
<th>Moderate No.</th>
<th>%</th>
<th>Focal No.</th>
<th>%</th>
<th>Negative No.</th>
<th>%</th>
<th>Kruskal-Wallis test</th>
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<td>4</td>
<td>6.4</td>
<td>1</td>
<td>25</td>
<td>1</td>
<td>25</td>
<td>1</td>
<td>25</td>
<td>1</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Steatohepatitic</td>
<td>5</td>
<td>8</td>
<td>1</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>2</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Pelioid</td>
<td>5</td>
<td>8</td>
<td>1</td>
<td>20</td>
<td>2</td>
<td>40</td>
<td>1</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>4</td>
<td>6.4</td>
<td>1</td>
<td>25</td>
<td>1</td>
<td>25</td>
<td>1</td>
<td>25</td>
<td>1</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>21</td>
<td>33.8</td>
<td>17</td>
<td>27.4</td>
<td>8</td>
<td>12.9</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total and % of positive cases</td>
<td>46 (74.19%)</td>
<td>25.8%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Table 3. The results of CK 19 regarding the histologic pattern of the primary HCC

<table>
<thead>
<tr>
<th>Pattern</th>
<th>No.</th>
<th>%</th>
<th>Strong No.</th>
<th>%</th>
<th>Moderate No.</th>
<th>%</th>
<th>Focal No.</th>
<th>%</th>
<th>Negative No.</th>
<th>%</th>
<th>Kruskal-Wallis test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trabecular</td>
<td>24</td>
<td>38.7</td>
<td>1</td>
<td>4.1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4.1</td>
<td>22</td>
<td>91.6</td>
<td>0.0014</td>
<td></td>
</tr>
<tr>
<td>Pseudo glandular</td>
<td>14</td>
<td>22.5</td>
<td>1</td>
<td>7.1</td>
<td>1</td>
<td>7.1</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>85.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid</td>
<td>6</td>
<td>9.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>16.6</td>
<td>5</td>
<td>83.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td>4</td>
<td>6.4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steatohepatitic</td>
<td>5</td>
<td>8</td>
<td>1</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelioid</td>
<td>5</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>4</td>
<td>6.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>3</td>
<td>4.8</td>
<td>3</td>
<td>4.8</td>
<td>2</td>
<td>3.2</td>
<td>54</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total and % of positive cases</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>12.9%</td>
<td></td>
<td></td>
<td>87.09%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. The results of Arg-1, HepPar-1 and CK 19 in the primary HCC

<table>
<thead>
<tr>
<th>Immunostaining</th>
<th>Strong No.</th>
<th>%</th>
<th>Moderate No.</th>
<th>%</th>
<th>Focal No.</th>
<th>%</th>
<th>Negative No.</th>
<th>%</th>
<th>Kruskal-Wallis test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg-1</td>
<td>23</td>
<td>37</td>
<td>20</td>
<td>27.4</td>
<td>12</td>
<td>12.9</td>
<td>7</td>
<td>11.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HepPar-1</td>
<td>21</td>
<td>33.87</td>
<td>17</td>
<td>27.4</td>
<td>8</td>
<td>12.9</td>
<td>16</td>
<td>25.8</td>
<td></td>
<td>0.92</td>
</tr>
<tr>
<td>CK 19</td>
<td>3</td>
<td>4.8</td>
<td>3</td>
<td>4.8</td>
<td>2</td>
<td>3.2</td>
<td>54</td>
<td>87.09</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. The results of Arg-1, HepPar-1 and CK 19 in the metastatic carcinoma to liver

<table>
<thead>
<tr>
<th>Immunostaining</th>
<th>Strong No.</th>
<th>%</th>
<th>Moderate No.</th>
<th>%</th>
<th>Focal No.</th>
<th>%</th>
<th>Negative No.</th>
<th>%</th>
<th>Kruskal-Wallis test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg-1</td>
<td>1</td>
<td>5.5</td>
<td>1</td>
<td>5.5</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>88.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HepPar-1</td>
<td>1</td>
<td>5.5</td>
<td>2</td>
<td>11.1</td>
<td>1</td>
<td>5.5</td>
<td>14</td>
<td>77.7</td>
<td></td>
<td>0.618</td>
</tr>
<tr>
<td>CK 19</td>
<td>5</td>
<td>27.7</td>
<td>6</td>
<td>33.3</td>
<td>2</td>
<td>11.1</td>
<td>5</td>
<td>27.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Panel summarizes the results of immunostaining of the three markers in the primary HCC and metastatic carcinoma

<table>
<thead>
<tr>
<th>Immunostaining</th>
<th>Primary HCC</th>
<th>Metastatic Carcinoma</th>
<th>Kruskal-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Arg-1+/HepPar-1+/CK 19+</td>
<td>5</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Arg-1-/HepPar-1+/CK 19+</td>
<td>2</td>
<td>3.2</td>
<td>1</td>
</tr>
<tr>
<td>Arg-1+/HepPar-1+/CK 19-</td>
<td>34</td>
<td>54.8</td>
<td>1</td>
</tr>
<tr>
<td>Arg-1+/HepPar-1-/CK 19+</td>
<td>14</td>
<td>22.5</td>
<td>0</td>
</tr>
<tr>
<td>Arg-1-/HepPar-1-/CK 19-</td>
<td>1</td>
<td>1.6</td>
<td>11</td>
</tr>
<tr>
<td>Arg-1-/HepPar-1+/CK 19-</td>
<td>1</td>
<td>1.6</td>
<td>3</td>
</tr>
<tr>
<td>Arg-1-/HepPar-1+/CK 19+</td>
<td>5</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td></td>
<td>18</td>
</tr>
</tbody>
</table>

Table 7. Detailed positive immunoreactivity for Arg-1, HepPar-1 and Ck 19 of liver metastases.

<table>
<thead>
<tr>
<th>Origin of Liver Metastases</th>
<th>No.</th>
<th>Arg-1</th>
<th>HepPar-1</th>
<th>Ck-19</th>
<th>Kruskal-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infiltrating duct carcinoma</td>
<td>4</td>
<td>22.2%</td>
<td>0%</td>
<td>0%</td>
<td>2% 50%</td>
</tr>
<tr>
<td>Lung adenocarcinoma</td>
<td>6</td>
<td>33.3%</td>
<td>0%</td>
<td>33.3%</td>
<td>5% 83.3%</td>
</tr>
<tr>
<td>Colon adenocarcinoma</td>
<td>6</td>
<td>33.3%</td>
<td>16.6%</td>
<td>16.6%</td>
<td>4% 66.6%</td>
</tr>
<tr>
<td>Pancreatic adenocarcinoma</td>
<td>2</td>
<td>11.1%</td>
<td>50%</td>
<td>50%</td>
<td>2% 100%</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>11.1%</td>
<td>22.2%</td>
<td>72.2%</td>
<td>P value 0.172</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td>22.2%</td>
<td>72.2%</td>
<td></td>
</tr>
</tbody>
</table>
Percentage of reactivity of Arg-1 in relation to histologic pattern

- Strong
- Moderate
- Focal
- Negative
Percentage of the reactivity of HepPar-1 in relation to histologic pattern
Percentage of the reactivity of CK 19 in relation to histologic pattern

- TRABECULAR
- PSEUDO GLANDULAR
- SOLID
- CLEAR CELL
- STEATOHEPATITIC
- PELIOID
- SARCOMATOID

Legend:
- Strong
- Moderate
- Focal
- Negative
The percentage of the panel of reactivity in both primary HCC and liver metastases.