ABSTRACT

BACKGROUND: Prognosis of advanced hepatocellular carcinoma (HCC) is still poor. In this retrospective study prognostic factors for long-term survival and an immunohistochemical panel for discrimination of HCC from other liver malignancies were analyzed.

MATERIALS AND METHODS: In 181 primary liver tumors clinical data, tumor characteristics and the primary mode of treatment were analyzed using univariate and multivariate statistics. In 156 cases (145 HCC, 36 intrahepatic CCC) the immunohistochemical profile of the tumor tissue using molecular markers as HepPar-1, AFP, CD34, CK7, CK20, CA19-9 and CDX2 was established routinely. Significance of marker expression, sensitivity, specificity and positive predictive value of the analyzed markers in relation to histological subtype were estimated using SPSS 10.0.

RESULTS: Median overall survival (OS) was 15 ± 19.2 months. Multivariate analysis identified tumor size ($p = 0.001$), grading ($p = 0.002$), proliferative activity (Ki67 level; $p = 0.032$), multifocal tumor ($p = 0.045$), liver function (Child-Pugh score, $p = 0.045$) and performed tumour resection ($p < 0.0001$) as independent prognostic factors for survival. HepPar-1 was the most frequently expressed marker in HCC (positive in 71.8%; $p < 0.0001$) whereas positive AFP staining was less common (positive in 48.7%; $p < 0.0001$). The CD34 protein as a marker for vascular-associated tissue showed a positive reaction in 54.1% of tissues from HCC patients in comparison to 2 patients (6%) with cholangiocarcinoma ($p < 0.0001$).

CONCLUSIONS: Our data identified tumor stage, tumor biology and performed surgical therapy as independent prognostic factors for OS in HCC. Best predictive markers for differentiation between HCC and CCC were HepPar-1, CK7 and CA19-9. Using this panel fast and accurate differentiation by IHC was possible in more than 95% of the patients.

Key words: Hepatocellular carcinoma; Ki67; proliferative activity; AFP; CK7; Immunohisto-chemistry (IHC)
INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer death worldwide. In the last few decades, the incidence of HCC has increased, possibly due to the growing worldwide prevalence of chronic hepatitis B or C. Other reasons for human hepatocarcinogenesis include alcohol abuse and metabolic disorders (e.g., hemochromatosis) leading to liver cirrhosis. Exposure to carcinogens (e.g., aflatoxin plays a minor role). Especially in developed countries, the emergence of hepatitis C virus (HCV) and chronic liver damage and inflammation due to steatohepatitis are increasingly prominent etiological factors for human hepatocarcinogenesis. Likewise, incidence of HCC increased in Germany up to 9.2-10.7/100,000 in men and 1.6-3.6/100,000 in women.

HCC is characterized by poor prognosis leading to the second most frequent cause of cancer-related mortality, and has the shortest survival time of any human cancer. Without therapy, patients with HCC normally die within 12 months, due to rapid progression. Currently, surgical resection and liver transplantation are the best available treatment options for HCC. Tumor resection including partial hepatectomy is widely accepted as the first treatment option for many HCC patients. Liver transplantation is significantly more laborious due to the lack of donor organs with long waiting periods, higher perioperative risk, and long-term immunosuppression. Despite surgical treatment options long-term prognosis of HCC is disappointing due to a high incidence of recurrence leading to 5 year overall survival rates below 30% in operated patients and in the range of 5-7% in patients without tumor resection. Tumor characteristics that determine the biological aggressiveness and metastatic potential of the disease may be important predictors of survival and hence important elements in the evaluation of HCC.

Differentiation of HCC from other liver tumors as cholangiocarcinoma (CCC) is in clinical routine a frequent diagnostic dilemma for pathologists. However, accurate diagnosis is crucial, because treatment options differ considerably: In contrast to CCC, HCC is chemo-resistant. Liver transplantation is a potential therapeutic option in patients with HCC and liver cirrhosis but usually not recommended in patients with CCC. In addition to hematoxylin and eosin (H&E) staining several immunohistochemical markers for distinction of liver tumors are used in clinical practice. However, utility of each of these markers is limited either by suboptimal sensitivity or difficulty in interpretation of results, especially in poorly differentiated tumors or cirrhotic hepatocellular carcinoma.

In this retrospective single center study we analyzed the impact of histopathological findings and of immunohistochemical markers for diagnosis and prognosis of HCC of different etiologies in a German patient population.

PATIENTS AND METHODS

Patients

Clinical and histopathological data of 145 patients with HCC (72.4% males; mean age 68.5 ± 9.9 years; for details see Table 1) and histopathological data of 36 patients with cholangiocarcinoma (CCC) were analyzed in this retrospective study. Our cohort includes patients who were admitted to the Marienhospital Stuttgart between January 2004 to February 2014.

Table 1 Clinical and etiological data of the study population (n = 181).

| Etiology                  | Gender (male %) | Mean age (years) | Hepatitis (HBV/HCV) (%) | Alcohol (%) | Steatohepatitis (%) | Cirrhosis (%) |
|---------------------------|-----------------|------------------|-------------------------|-------------|---------------------|--------------|
| Cholangiocarcinoma (n = 36) | 30              | 67.5 ± 11.5      | 5.4                     | 8.1         | 8.1                 | 13.5         |
| Hepatocellular carcinoma (n = 145) | 72.4           | 68.5 ± 9.9       | <0.001                  | <0.001      | n.s.                | < 0.001      |
| p-value                   | 0.048           | n.s.             | <0.001                  | <0.001      | n.s.                | < 0.001      |
RESULTS

Association of tumor therapy, HCC stage (TNM classification), and liver function with overall survival (OS)
Median overall survival (OS) of all patients was 15 ± 19.2 months. OS correlated significantly with treatment of HCC. After surgical therapy OS was significantly longer (24.5 ± 28.9 months) than in patients without tumor resection (6.5 ± 9.6 months, \( p < 0.0001 \)). OS in patients with small tumor size (T1) was significantly longer (36 ± 22.3) in comparison to patients with T4 tumors with OS of only 7 ± 6.9 months \(( p < 0.0001)\). Following the 2-year observation period 61.7% of patients with T1 tumors were still alive whereas all patients with T4 tumors had died (Figure 1). After 5 year follow-up the overall survival rates of patients with T1 tumor dropped down to 34.8%. In patients with advanced liver disease and reduced liver function (Child-Pugh B/C) OS dropped significantly after 2 years as compared to patients with Child-Pugh A liver function (8.5% vs 48.4%; \( p < 0.001 \)).

Association of tumor differentiation with tumor stage (TNM), multifocal tumor and proliferative activity (Ki67)
Tumor differentiation correlated significantly with tumor stage and proliferative activity. In larger tumors (T3, T4), incidence of poorly differentiated tumor cells was 31.1% and 81.2%, whereas in T1 and T2 tumors only 0% or 8.3% dedifferentiated tumor cells were detected \(( p = 0.0037; \) see Figure 2). Tumor cell differentiation was also significantly associated with multifocal HCC nodes (45.5% in G3 tumors vs 17.6% in G1 tumors; \( p = 0.0003 \)). In G1 tumors Ki67 labelling was significantly lower \((12.7 ± 17.6%; \) see Figure 3).

Multivariate analysis of prognostic factors in HCC
Multivariate analysis identified tumor size (T stage; \( p = 0.001 \)), grading \(( p = 0.002 \)), proliferative activity (Ki67 level; \( p = 0.032 \)), liver function (Child-Pugh score; \( p = 0.045 \)), multifocal tumor stage \(( p = 0.045 \)) and performed tumor resection \(( p < 0.0001 \)) as independent prognostic factors for survival.

Immunophenotypic Profile of Hepatocellular Carcinoma
HepPar-1 positive in 71.8% of HCCs and was the most frequently expressed marker in this disease. In CCC the marker was detectable in 3% only \(( p < 0.0001 \)). AFP staining was positive in 71 HCCs \((48.7%; \) \( p < 0.0001 \)) and also in one patient with CCC. In this patient histological examination showed a combined hepatocellular-cholangiocarcinoma (combined HCC-CCC) with > 50% of malignant cholangiocytes. The CD34 protein as a marker for vascular-associated tissue showed a positive reaction in 65 patients \((54.1\%) \) with HCC in comparison to 2 patients \((6\%) \) with cholangiocarcinoma \(( p < 0.0001 \)).

31 patients \((86.9\%) \) with intrahepatic cholangiocarcinoma expressed CK7 and 18 \((50\%) \) expressed CK20. These results were significantly different from classical hepatocellular carcinoma (CK7 and CK20 expression in 24.3% and 0%, respectively; \( p < 0.001 \) and \( p < 0.0001 \)). Except for one patient with combined HCC-CC (see above) AFP and HepPar-1 were negative in all patients with HCC.
For more details see Table 2, Figure 4 and Figure 5a, b.

**Serum levels of tumor marker AFP and correlation with IHC**

In 36% of patients with HCC serum tumor marker AFP was significantly elevated. When compared with the AFP staining in the liver a significant correlation between IHC and serum AFP levels was detected. Increased serum AFP was detectable in only 14.3% of the patients without AFP expression in IHC. Mean AFP serum concentration in these patients was 105 ± 315 ng/mL. In HCC with positive AFP expression in IHC, serum levels were significantly elevated in 63.6% (p < 0.001) with a mean serum level of 2129 ± 1661 ng/mL (see Figure 5c).

**DISCUSSION**

In the present study, overall survival of 145 patients with HCC was analyzed in relation to different risk factors for tumor recurrence and cancer-related death. In addition, an immunohistochemical panel was examined to differentiate HCC from CCC.

Despite new diagnostic approaches and novel therapeutic modalities as tyrosine kinase inhibitors, the prognosis of advanced HCC still remains poor. In our study, overall survival median OS
was 15.0 ± 19.2 months. Whereas in other human cancers prognosis usually depends on tumor stage and aggressiveness survival of most patients with liver cancer is also affected by the underlying chronic liver disease (i.e. cirrhosis and reduced liver function). As shown previously, reduced liver function and end-stage liver cirrhosis (in most cases documented using Child-Pugh or BCLC score) correlate with OS and are a significant predictors for survival of patients with HCC[22,23]. In our study, OS was also significantly lower in patients with reduced liver function (Child B/C) when compared with patients with nonrestrictive liver function (Child-Pugh A), probably due to the fact that only a minority of patients (32.2%) had surgical treatment. It is not surprising that tumor stage at diagnosis is one of the strongest prognostic factors since R0 resection or liver transplantation is the only treatment options offering long-term survival or cure. Possibly due to the underlying liver disease 5-year OS dropped down to 34.8% even in the group of T1 patients with primarily favourable prognosis. As demonstrated earlier[24] multifocal tumor stage was another adverse prognostic indicator of overall survival (7.5 ± 9.1 months; \( p = 0.012 \)), perhaps due to the underlying liver disease, e.g. cirrhosis, favouring multicentre occurrence[25].

Dysregulation of the balance between proliferation and cell death represents a pro-tumorigenic principle in human carcinogenesis resulting in tumor progression and tumor cell seed with occurrence of metastasis. The Ki67 protein is associated with active cell proliferation and expressed in all phases of the cell cycle, especially in G2/M, except for G0. In our study high proliferative activity (measured by Ki67 labelling index) was significantly correlated with poor tumor cell differentiation (\( p < 0.0001 \)). Similar results were seen in previous studies showing higher levels of Ki67 expression in tumor tissue to be associated with higher tumor grade[26-28] and early disease recurrence[22] probably as a consequence of tumor evolution and higher tumor aggressiveness[29,30]. Histopathologic and biologic factors of tumor such as tumor size (\( p = 0.001 \)) and multifocal tumor stage (\( p = 0.045 \)), surgical treatment (\( p < 0.0001 \)), liver function (\( p = 0.045 \)), cell differentiation (\( p = 0.002 \)) and proliferative activity (\( p = 0.032 \)) were detected as independent prognostic factors using multivariate analysis for OS in patients with HCC.

Diagnosis of Hepatocellular carcinoma (HCC) may be difficult especially in well and poorly differentiated HCC. In well differentiated tumors distinction from normal or regenerative tissue may be very difficult in some cases, whereas some of the unusual morphologic variants, including clear-cell, pleomorphic, and sarcomatoid variants or poorly differentiated tumors, may be mistaken for metastases[29-31].

In our study all tumors could be differentiated by immunohistochemistry, but sometimes only after a second look using the whole hepatobiliary panel and also taking into account clinical reports and serum markers such as AFP or CA19-9. In our cohort typical risk factors for HCC such as viral hepatitis, alcohol abuse, underlying liver cirrhosis and male sex were represented significantly more often in patients with HCC in comparison to the group with cholangiocarcinoma. Thus clinical data and pathologic often depend well with the results of HCC but, again, sensitivity was low for detection of HCC. Similar data of a low sensitivity (20-60%) and better specificity (76-96%) of AFP in HCC were published earlier[32,33] leading to the current recommendation by the German Association for the Study of the Liver (GASL)[34] not to use AFP for screening of HCC. HepPar-1 is a very important immunohistochemical marker for HCC and our data are in line with previous studies reporting high expression levels of HepPar-1 in primary liver cancer[35,36] However, in HCC with scirrhouss morphology, absence of HepPar-1 staining and frequent positivity of adenocarcinoma-related markers is characteristic and needs to be differentiated from liver metastasis. IHC for CK7 was positive in more than 24% of our patients with HCC. Interestingly, since it is known that CK7 is expressed in hepatic progenitor cells (HPCs) but normally not in hepatocytes, CK7 positive HCCs potentially derive from HPCs[37]. CK7 is of particular importance to distinguish scirrhouss from fibrolamellar HCC, the latter expressing this marker much more often[38,39]. In comparison to CK7, CK20 was expressed only in CCC and thus is helpful to distinguish this tumor from HCC. However, since sensitivity of this marker for CCC is low and since there is stronger expression in colorectal cancer, there may be a pitfall in differentiation of primary liver cancer against secondary tumors of the liver[37]. To solve this problem in clinical routine another immunohistochemical marker with high sensitivity and specificity for malignant hepatocytes Glypican-3 (GPC-3) is currently available; however, this was not the case in our retrospective study. GPC-3 is a membrane-anchored heparin sulfate proteoglycan that has been shown to be expressed in approximately 80% of HCC but not in benign hepatic lesions[39-41]. This antigen may also represent a potential therapeutic target[40-41].

There are several limitations to our study. It is a retrospective study and patients with variable treatment modalities, liver function and different etiologies of HCC were included. In most cases (67.8%) histological diagnosis was performed by a needle biopsy only leading to uncertainties in the determination of precise biological characteristics of the tumors, e.g. proliferative activity and tumor cell differentiation neglecting also intraspecific heterogeneity in larger HCCs. Due to the retrospective nature of this study, Child-Pugh score could be estimated in only 78 patients of our cohort.

Our findings are in line with earlier studies[42,43] regarding prognostic factors HCC and emphasize the importance of tumor stage, tumor biology and liver function for long-term survival of these patients. In addition, the present study confirms earlier reports on the complexity of making an accurate diagnosis of HCC and cholangiocarcinoma. For reliable differentiation of primary liver cancer a panel of immunohistochemical markers such as HepPar-1, AFP, CK7 and CA19-9 is essential together with clinical data. Gene expression analysis may open novel perspectives to find better diagnostic and prognostic markers and potential therapeutic targets for primary liver cancer[44,45]. Early data indicate that gene expression profiling may be helpful for subclassification of HCC, cholangiocellular carcinoma and especially combined hepatocellular-cholangiocarcinoma in future clinical routine[46].

REFERENCES

1. Tsukuma H, Hiya1ma T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakashima K, Fujimoto I, Inoue A, Yamazaki H: Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993; 328: 1797–1801. [PMID: 7648822]
2. World Health Organization. Mortality database. 2010; Available from: www.who.int/whosis/en.
3. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007; 132: 2557-2576. [PMID: 17570226]; [DOI: 10.1053/j.gastro.2007.04.061]
4. Gomaa AI, Khan SA, Toledano MB, Waked I, Taylor-Robinson SD. Hepato-cellular carcinoma: epidemiology, risk factors and
pathogenesis. *World J Gastroenterol* 2008; 14: 4300-4308. [PMID: 18666317]
5. Okuda K. Hepatocellular carcinoma. *J Hepatol* 2000; 32: 225-237. Review. [PMID: 10728807]
6. Ferlay J, Shin H, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; 127: 2893-2917. [PMID: 21351269]; [DOI: 10.1002/ijc.25516]
7. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *Carcinogenesis* 2011; 61: 69-90. [PMID: 21296855]; [DOI: 10.1033/caac.20107]
8. Forner A, Reig ME, de Lope CR, Bruix J. Current strategy for staging and treatment: the BCLC update and future prospects. *Semin Liver Dis* 2010; 30: 61-74. [PMID: 20175034]; [DOI: 10.1055/s-0030-1247133]
9. El-Serag HB, Marrero JA, Rudolph L, Reddy KR. Diagnosis and treatment of hepatocellular carcinoma. *Gastroenterology* 2008; 134: 1752-1763. [PMID: 18471552]; [DOI: 10.1053/j.gastro.2008.02.090]
10. Roayaie S, Obiedat K, Sposito C, Mariani L, Bhooi S, Pellegrinelli A, Labow D, Llovet JM, Schwartz M, Mazzaferrro V. Resection of hepatocellular cancer ≤2 cm: results from two Western centers. *Hepatology* 2013; 57: 1426-1435. [PMID: 22576353]; [DOI: 10.1002/hep.25832]
11. Yi NJ, Suh KS, Kim T, Kim J, Shin WY, Lee KU. Current role of surgery in treatment of early stage hepatocellular carcinoma: resection versus liver transplantation. *Oncology* 2008; 75: 124-128. [PMID: 19092282]; [DOI: 10.1159/0001073434]
12. Poon RT, Fan ST, Lo CM, Liu CL, Yuen KM, Lam MS, Rizzato F, Sjaastad O, Wong J. Long-term survival and pattern of recurrence after resection of small hepatocellular carcinoma in patients with preserved liver function: implications for a strategy of salvage transplantation. *Ann Surg* 2002; 235: 373-382. [PMID: 11882759]
13. Kaibori M, Ishizaki M, Saito T, Matsui K, Kwon AH, Kamiyama Y. Risk factors and outcome of early recurrence after resection of small hepatocellular carcinomas. *Am J Surg* 2009; 198: 39-45. [PMID: 19178896]; [DOI: 10.1016/j.amjsurg.2008.07.051]
14. Taketomi A, Toshima T, Kitagawa D, Motomura T, Takeishi K, Seta T, Ohno K, Kaneko S, Ohira K, Hayashi M, Ohno Y, Takemoto Y, Yamasaki A, Hayashi T, Chang YC, Kohno H, Nakamura T, Yuki H. Incidence and factors associated with intrahepatic recurrence following resection of hepatocellular carcinoma. *Gastroenterology* 1993; 105: 488-494. [PMID: 8392955]
15. Koskinas J, Petritaki K, Kavantzas N, Rapti I, Kountouras D, Hadziyannis S. Hepatic expression of the proliferative marker Ki-67 and p53 protein in HBV or HCV cirrhosis in relation to dysplastic liver cell changes and hepatocellular carcinoma. *J Viral Hepat*. 2005; 12: 635-43. [PMID: 16255765]; [DOI: 10.1111/j.1365-2893.2005.00635.x]
16. D’Errico A, Gregioni WF, Fiorentino M, Grazi GL, Mancini AM. Overexpression of p53 protein and Ki-67 proliferative index in hepatocellular carcinoma: an immunohistochemical study on 109 Italian patients. *Pathol Int.* 1994; 44: 682-687. [PMID: 7804430]
17. Yeh MM, Larson AM, Campbell JS, Fausto N, Ruyuk SJ, Swanson PE. The expression of transforming growth factor-alpha in cirrhosis, dysplastic nodules, and hepatocellular carcinoma: an immunohistochemical study of 70 cases. *Am J Surg Pathol* 2007; 31: 681-689. [PMID: 17460450]; [DOI: 10.1097/PAS.0b013e31802ff7aa]
18. Quaglia A, McStay M, Stoeber K, Loddo M, Caplin M, Fanshawe T, Williams G, Dhillon A. Novel markers of cell kinetics to evaluate progression from cirrhosis to hepatocellular carcinoma. *Liver Int* 2006; 26: 424-432. [PMID: 16629645]; [DOI: 10.1111/j.1478-3231.2006.01242.x]
19. Koduko N, Nakamura M. Evidence-based clinical practice guidelines for patients undergoing curative resection of hepatocellular carcinoma: a new stratification of Barcelona Clinic Liver Cancer stage C: results from a French multicenter study. *Eur J Gastroenterol Hepatol*. 2016; 28: 433-440. [PMID: 26695429]; [DOI: 10.1097/MEG.0000000000000558]
32. Deutschen Krebsgesellschaft e.V. Diagnostik und Therapie des Hepatozellulären Karzinoms. Version 1.0 – März 2013. AWMF-Registernummer: 032/053OL
33. Wang L, Vuolo M, Suhrland MJ, Schlesinger K, HepPar1, MOC-31, pCEA, mCEA and CD10 for distinguishing hepatocellular carcinoma vs. metastatic adenocarcinoma in liver fine needle aspirations. Acta Cytol 2006; 50: 257-62. [PMID: 16780018]
34. Durnez A, Verslype C, Nevens F, Desmet V, Roskams T. The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. Histopathology. 2006; 49: 138-51. [PMID: 16879391]; [DOI: 10.1111/j.1365-2559.2006.02468.x]
35. Abdul-Al HM, Wang G, Makhlouf HR, Goodman ZD. Fibrolamellar hepatocellular carcinoma: an immunohistochemical comparison with conventional hepatocellular carcinoma. Int J Surg Pathol 2010; 18: 313-8. [PMID: 20444731]; [DOI: 10.1177/1066896910364229]
36. Ward SC, Waxman S. Fibrolamellar carcinoma: a review with focus on genetics and comparison to other malignant primary liver tumors. Semin Liver Dis 2011; 31: 61-70. [PMID: 21344351]; [DOI: 10.1055/s-0031-1272835]
37. Maeda T, Kawai T, Hasegawa K, Sugimachi K, Tsuneyoshi M. The expression of cytokeratin 7, 19, and 20 in primary and metastatic carcinomas of the liver. Mod Pathol 1996; 9: 901-9. [PMID: 8878022]
38. Krings G, Ramachandran R, Jain D, Wu TT, Yeh MM, Torbenson M, Kakar S. Immunohistochemical pitfalls and the importance of glypican 3 and arginase in the diagnosis of scirrhous hepatocellular carcinoma. Mod Pathol 2013; 26: 782-91. [PMID: 23348905]; [DOI: 10.1038/modpathol.2012.243]
39. Nassar A, Cohen C, Siddiqui MT: Utility of glypican-3 and survivin in differentiating hepatocellular carcinoma from benign and preneoplastic hepatic lesions and metastatic carcinomas in liver fine-needle aspiration biopsies. Diagn Cytopathol. 2009; 37: 629-35. [PMID: 19405109]; [DOI: 10.1002/dc.21075]
40. Feng M, Kim H, Phung Y, Ho M. Recombinant soluble glypican 3 protein inhibits the growth of hepatocellular carcinoma in vitro. Int J Cancer 2011; 128: 2246-7. [PMID: 20617511]; [DOI: 10.1002/ijc.25549]
41. Filmus J, Capurro M. Glypican-3: a marker and a therapeutic target in hepatocellular carcinoma. FEBS J 2013; 280: 2471-6. [PMID: 23305321]; [DOI: 10.1111/febs.12126]
42. Worns MA, Bosslet T, Victor A, Koch S, Hoppe-Lotichius M, Heise M, Hansen T, Pinot MB, Niederle IM, Schuchmann M, Weinmann A, Dührer C, Galle PR, Otto G. Prognostic factors and outcomes of patients with hepatocellular carcinoma in non-cirrhotic liver. Scand J Gastroenterol 2012; 47: 718-728. [PMID: 22472070]; [DOI: 10.3109/00365521.2012.677952]
43. Mao YM, Luo ZY, Li B, Hu TY. Prospective study on the survival of HCC patients treated with transcatheter arterial lipiodol chemoembolization. Asian Pac J Cancer Prev 2012; 13: 1039-1042. [PMID: 22631634]
44. Hass HG, Jobst J, Scheurlem M, Vogel U, Nehls O. Gene expression analysis for evaluation of potential biomarkers in hepatocellular carcinoma. Anticancer Res 2015; 35: 2021-8. [PMID: 25862856]
45. Andrisani OM, Studach L, Merle P. Gene signatures in hepatocellular carcinoma (HCC). Semin Cancer Biol. 2011; 21: 4-9. [PMID: 20851183]; [DOI: 10.1016/j.semcancer.2010.09.002]
46. Wang L, Zang W, Xie D, Ji W, Pan Y, Li Z, Shen J, Shi Y. Comparison of hepatocellular carcinoma (HCC), cholangiocarcinoma (CC), and combined HCC-CC (CHC) with each other based on microarray dataset. Tumour Biol 2013; 34: 1679-84. [PMID: 23532688]; [DOI: 10.1007/s13277-013-0702-6]

Peer reviewers: Hoe Bok Chae; Mohamed Hassany; Cerwenka Herwig