Increased Alpha-Fetoprotein Serum Level is Predictive for Survival and Recurrence of Hepatocellular Carcinoma in Non-Cirrhotic Livers

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Key Words

Hepatocellular carcinoma · Liver · Non-cirrhotic liver · Survival · Non-viral factors · Risk factors

Abstract

Background: Hepatocellular carcinoma (HCC) may be diagnosed in the absence of cirrhosis. However, little is known about prognostic factors for the survival of HCC patients with a non-cirrhotic liver in the absence of well-established risk factors. **Method:** Survival rates and risk factors for survival and recurrence were analysed in all patients diagnosed between 2000 and 2010 with HCC in a non-cirrhotic liver and in the absence of well-established risk factors. **Results:** Ninety-four patients were analysed. Treatment with curative intent consisted of surgical resection in 43 patients (46%) and radiofrequency ablation in 4 patients (4%). In patients treated with curative intent and alive 30 days after treatment (n = 40), 1- and 5-year overall survival rates were 95 and 51%, respectively. Patients with a high preoperative α -fetoprotein (AFP) serum level, the presence of microvascular invasion in the resected specimen, a complicated postoperative course and a major resection, due to a great-

er tumour volume, had a significantly worse outcome and a higher recurrence rate. In multivariate analysis, a high AFP serum level at presentation was significantly associated with recurrence and a worse survival. **Conclusion:** HCC presenting in a non-cirrhotic liver in the absence of well-established risk factors has a poor prognosis. Increased AFP serum levels are significantly associated with clinical outcome.

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer-related mortality [1]. The incidence of HCC is high in Asia and parts of Africa, whereas it is low but increasing in the Western world, even in the Netherlands [1–5].

HCC is the most common primary tumour of the liver and typically develops in the presence of underlying cirrhosis, particularly in high-endemic areas [1]. In low-endemic areas, no cirrhosis is diagnosed in 5–40% of HCC patients [6–10]. Although many investigators have re-

ported on prognostic factors for HCC in a non-cirrhotic liver, they still included patients positive for serological markers of hepatitis B or C virus infection, alcohol abuse or haemochromatosis. These are known well-established risk factors for the development of cirrhosis and eventually HCC [11].

In HCC patients with no underlying cirrhosis, the 'normal' quality of the non-tumoural liver parenchyma might make this type of HCC a different entity, with a different aetiology, clinical presentation, management and prognosis [12].

The aim of this retrospective study was to assess the clinical presentation, surgical outcome and prognostic factors in our series of patients who had HCC without the presence of underlying cirrhosis or well-established risk factors for cirrhosis and HCC.

Methods

Clinical Characteristics

All patients with HCC diagnosed in a tertiary reference centre between January 2000 and December 2010 were analyzed. Patients included ($n = 94$) had no underlying cirrhosis and none of the well-established risk factors for cirrhosis or HCC as determined by negative hepatitis B and C serological markers (anti-hepatitis C virus negative, hepatitis B surface antigen negative, anti-hepatitis B core negative), a negative history of alcohol abuse (more than 6 glasses of alcohol once a week [13]) and absence of haemochromatosis or a metabolic liver disease. Preoperatively, HCC was diagnosed by means of imaging criteria using 2 imaging techniques. If HCC was diagnosed, treatment was discussed in a multidisciplinary meeting according to the American Association for the Study of Liver Diseases guidelines [1]. In all patients, α -fetoprotein (AFP) serum levels were assessed. The AFP cut-off point for our hospital is 9 ng/ml. Data on age, gender, size and number of lesions, and the presence and extent of extrahepatic disease were collected.

Patients were primarily assessed for surgical resection; those not amenable for this option were considered for radiofrequency ablation (RFA). Treatment with curative intent was defined as a complete surgical resection, including wedge resection, segment resection, hemihepatectomy and RFA. The presence of a conventional HCC in a non-cirrhotic liver was histologically confirmed postoperatively in all patients. Patients with a fibrolamellar HCC were excluded. Tumour differentiation grade was determined according to the Lauwers classification [14].

Postoperative complications were reported up to 30 days following surgery and were grouped as complicated (pharmacological treatment or intervention needed) or uncomplicated. Patients not amenable for curative therapy were offered palliative options, consisting of either transarterial chemo-embolization, radiotherapy, palliative systemic therapy or best supportive care.

Follow-Up

After surgical resection, patients were followed up using contrast-enhanced computed tomography or magnetic resonance imaging combined with determination of serum AFP at

3- and 6-month intervals for up to 2 years following treatment. After 2 years, follow-up was continued annually for up to 5 years. HCC recurrence was defined as the appearance of a new focal liver lesion with HCC characteristics, enlargement of lymph nodes in the liver hilum or suspected extrahepatic lesions. The diagnosis was histologically confirmed if necessary. Data regarding survival of patients who were lost to follow-up was obtained from the general practitioner or the civil registration systems.

Statistical Analysis

Variables were compared using the Mann-Whitney U test, t test or χ^2 test, as appropriate. Statistical significance was considered at a p value <0.05 . Receiver operating characteristic (ROC) plots were used to define the optimum cut-off point for serum AFP. Multivariate Cox regression analysis was performed to assess the independent prognostic factors. According to the literature, the patient characteristic 'recurrence of disease' has a high negative impact on survival, therefore the analysis was performed within a model adjusting for recurrence. Within this model, preoperative biochemical and postoperative tumour characteristics with a p value <0.05 in univariate analysis together with the baseline characteristics age and gender were included to determine the independent contribution of each variable. Survival curves were drawn using the Kaplan-Meier method. Differences between Kaplan-Meier curves were tested using the log-rank test. All analyses were performed using the Statistical Package for Social Sciences 17.0 (SPSS, Chicago, Ill., USA).

Results

Patient Characteristics

Ninety-four patients with HCC without underlying cirrhosis and with no established risk factors for HCC were identified. Of these, 46 patients (49%) were female and the median age was 62 years (range 10–87); median BMI was 23 (range 18–44), and in 12 patients (13%), diabetes mellitus was present. Table 1 summarizes the patients' biochemical and radiological characteristics.

Outcome

Forty-three patients (46%) underwent a surgical resection and 4 (4%) underwent RFA. Out of 43 patients who underwent a surgical resection, 3 patients died within 30 days after surgery; 2 patients died due to a myocardial infarct and 1 patient died because of an eventful postoperative course secondary to bleeding. Median time to death was 44 months (range <1 –128).

After complete surgical resection ($n = 40$) of a histologically confirmed conventional HCC in a non-cirrhotic liver, the 1-, 3- and 5-year survival rates were 95, 60 and 51%, respectively. Of the 17 patients with an increased serum AFP (>9 ng/ml), 13 (76%) had a normalized serum

Table 1. Biochemical and radiological characteristics of HCC patients without well-established risk factors

Characteristic	Reference value	HCC patients of our hospital
Albumin, g/l	35–50	41 (27–52)
ASAT, U/l	0–34	50 (16–897)
ALAT, U/l	0–44	40 (6–461)
AP, U/l	0–114	159 (55–2,024)
Bilirubin, μ mol/l	0–16	10 (4–199)
AFP, μ g/l	0–9	12 (1–834,350)
Number of tumours		1 (1–7)
Radiological tumour size, mm		98 (5–250)
TNM		
Stage I		28 (30)
Stage II		16 (17)
Stage IIIA		12 (13)
Stage IIIB		6 (6)
Stage IV		32 (34)

Data are expressed as medians (range) or numbers of patients (%). ASAT = Aspartate aminotransferase; ALAT = alanine aminotransferase; AP = alkaline phosphatase.

AFP after surgical resection. The 4 patients without a normalized serum AFP had HCC metastasis within 5 months after surgery. Fifteen out of 40 patients (38%) treated by surgical resection had recurrence of HCC during follow-up. Median time to recurrence was 8 months (range 1–20). Of the patients treated with a minor resection ($n = 12$), 3 patients had recurrence of HCC. In the case of wedge resections, HCC recurrence was found in the ipsilateral hemi-liver.

Table 2 presents the operative characteristics and post-operative histological characteristics of the patients treated with surgical resection; no characteristics suspicious for hepatocellular adenoma were found.

In 47 patients (50%) not amenable for curative treatment, palliative treatment was considered. They received either transarterial chemo-embolization/transarterial embolization (3 patients; 3%), radiotherapy or systemic chemotherapy (14 patients; 15%) or best supportive care (30 patients; 32%).

Factors Associated with Survival and Recurrence of HCC after Surgical Resection

The optimal AFP cut-off point selected by ROC plot in 40 patients treated with a surgical resection was between 8.0 and 9.5 ng/ml. Patients with a poor survival after surgical resection more often underwent a major resection, had the presence of microvascular invasion, a moderately

Table 2. Surgical and pathological parameters in HCC patients undergoing surgical resection ($n = 40$)

Parameter	
<i>Type of resection</i>	
Minor resection	
Segment	11 (28)
Major resection	
Left hemihepatectomy	10 (25)
Right hemihepatectomy	16 (40)
Extended left hemihepatectomy	1 (2)
Extended right hemihepatectomy	2 (5)
Postoperative mortality and morbidity	
Uncomplicated	25 (63)
Complicated	15 (37)
Fibrosis	
F0	19 (48)
F1	17 (42)
F2	4 (10)
Steatosis	
G0	22 (55)
G1	15 (38)
G2	2 (5)
G3	1 (2)
Microvascular invasion	
Presence	22 (55)
Absence	17 (43)
Undefined	1 (2)
Differentiation grade	
Good	8 (20)
Moderate	22 (55)
Poor	8 (20)
Undefined	2 (5)

Data are expressed as numbers of patients, with percentages in parentheses.

or poorly differentiated tumour, a complicated postoperative course, an increased preoperative serum AFP and recurrence of disease (table 3). After adjusting for recurrence, in the Cox regression analysis an increased preoperative AFP serum level was shown to be significantly associated with a worse survival (table 4a).

Patients with recurrence of HCC were characterized by higher preoperative AFP serum levels ($p = 0.003$) and the presence of microvascular invasion ($p = 0.005$).

In the Cox regression analysis, an increased preoperative AFP serum level and the presence of microvascular invasion were shown to be significantly associated with HCC recurrence (table 4b).

The 1- and 3-year survival rates of patients with a high and low AFP serum level were 53 and 86%, and 21 and 75%, respectively ($p = 0.010$; fig. 1).

Table 3. Biochemical, radiological, surgical and pathological characteristics associated with overall survival following surgical resection (n = 40; univariate analysis)

Characteristic	Survivors	Non-survivors	p value
AFP			0.010
≤9 ng/ml	74	26	
>9 ng/ml	47	53	
TNM			0.345
Stage I/II	62	38	
Stage III	62	38	
Number of tumours			0.134
1	59	41	
>1	83	17	
Radiological tumour size			0.514
≤5 mm	80	20	
5–10 mm	56	44	
>10 mm	57	43	
Postoperative course			0.008
Uncomplicated	72	28	
Complicated	50	50	
Type of resection			0.030
Minor	82	18	
Major	55	45	
Fibrosis			0.512
F0	68	32	
F1	53	47	
F2	75	25	
Steatosis			0.176
G0	55	45	
G1	63	27	
G2	50	50	
G3	100	0	
Microvascular invasion (histology)			0.035
Presence	55	45	
Absence	76	24	
Differentiation grade (histology)			0.048
Good	87	13	
Moderate	59	41	
Poor	40	60	
Recurrence			<0.001
Present	13	87	
Absent	82	18	

Data are expressed as percentages.

Fig. 1. Overall survival of HCC patients without well-established risk factors starting at 30 days after surgical resection comparing normal versus increased serum levels of AFP.

Table 4. Prognostic factors associated with overall survival and recurrence in multivariate analysis (n = 40)

a Prognostic factors associated with overall survival

Characteristic	Hazard ratio	p value
Age (years)	0.98 (0.94–1.02)	0.263
Gender	0.41 (0.80–2.07)	0.280
AFP (ng/ml) ¹	1.21 (1.02–1.45)	0.034
Differentiation grade (good vs. moderate vs. poor)	1.98 (0.45–8.72)	0.370
Microvascular invasion (absent vs. present)	3.55 (0.69–18.33)	0.130

b Prognostic factors associated with recurrence

Characteristic	Hazard ratio	p value
Age (years)	0.97 (0.93–1.01)	0.120
Gender	1.29 (0.39–4.65)	0.702
AFP (ng/ml) ¹	1.29 (1.02–1.63)	0.031
Differentiation grade (good vs. moderate vs. poor)	1.05 (0.21–5.10)	0.957
Microvascular invasion (absent vs. present)	7.03 (1.03–47.97)	0.047

Values in parentheses represent 95% confidence limits.

¹ Continuous variable.

Discussion

In this study, we evaluated the outcome of HCC treatment in patients without well-established risk factors for cirrhosis or HCC. Several studies have explored clinicopathological characteristics in HCC patients without cirrhosis [1, 11, 15]. Although they studied HCC in the absence of cirrhosis, they often included patients with well-established risk factors for cirrhosis or HCC or analyzed a small group of patients [11, 16]. This selection bias will influence overall survival as it is known that, for example, chronic hepatitis B influences survival in HCC patients [17]. We were able to include a large group of patients, all with non-cirrhotic HCC without well-established risk factors, and had the opportunity to study this unique population.

Diagnosis

In the absence of a screening program developed for patients with liver cirrhosis, early detection in an asymptomatic stage is seldom seen, and diagnosis of non-cirrhotic HCC is usually made at an advanced stage with large tumours [18]. By then, many patients have already developed an unresectable tumour, either because of tumour size, anatomical localization or the presence of multiple bilobar intrahepatic metastases [19]. In the present study, nearly 50% of the patients referred with HCC without liver cirrhosis and well-established risk factors were beyond curative surgical options. The studied population was homogenous for conventional HCC; none of the patients had a fibrolamellar HCC.

Survival and Recurrence

In our population of HCC patients without well-established risk factors, the survival rates were comparable with those of patients with Child-Pugh A liver cirrhosis treated with surgical resection (5-year survival rate 50%), with similar associated factors [1, 20]. As the optimal AFP cut-off point selected by ROC plot was between 8.0 and 9.5 ng/ml, the AFP cut-off point of our hospital (9 ng/ml) was an adequate cut-off point. Although AFP as a serological marker for HCC in the screening and surveillance of patients with cirrhosis is debated [21], AFP seems a useful marker in patients with HCC without well-established risk factors, especially when the AFP serum level is increased. It is likely that the 4 patients without normalization of AFP who had metastases within 5 months of surgery already had metastatic disease at surgery that was not identified by imaging due to small size.

According to the literature, little is known about the value of the AFP serum level in patients with HCC without well-established risk factors. Our study displays the presence of an increased AFP serum level preoperatively to be the most important factor associated with a worse outcome and recurrence of HCC in multivariate analysis. If the AFP serum level is increased in patients who have to undergo a high-risk operation, e.g. an extended hemihepatectomy, or in patients with many comorbidities, it might be worth reconsidering the high-risk treatment.

Therapy Considerations

If a surgical resection is performed with curative intent in a non-cirrhotic liver, 38% of patients suffer from recurrence of disease. Solitary recurrence might benefit from repeated resection, but in most patients recurrence after primary resection will be multifocal because of intrahepatic dissemination from the primary tumour [1]. Out of 15 patients with recurrence after treatment with curative intent, 3 had recurrence after a local surgical resection. Their disease recurrence appeared as a tumour in the ipsilateral hemi-liver, whereas the contralateral hemi-liver was free of tumour recurrence. Thus, in HCC patients with a well-preserved liver function, there might be a preference to perform a major resection because, after minor resections, patients tend to more often have recurrence in the ipsilateral hemi-liver. In the absence of well-established risk factors, the presence of a small satellite tumour, too small to detect on imaging, might cause tumour recurrence in the ipsilateral liver lobe.

Until now, liver transplantation has been a therapy considered for cirrhotic patients only. In patients with underlying cirrhosis treated with liver transplantation, 5-year survival rates up to 70% are reported [1]. This higher survival rate makes transplantation a treatment option worth considering for HCC patients without well-established risk factors [19]. A recent study by Mergental et al. [22] reported the largest series of patients transplanted for non-cirrhotic HCC. After analysing 105 patients, they concluded that liver transplantation may be justified as a curative treatment option in a selected group of patients with unresectable HCC in an otherwise normal liver [22]. The Milan criteria and the algorithms used for HCC in cirrhotic livers are probably not applicable to HCC in a non-cirrhotic liver without well-established risk factors, because it is a different disease with (probably) different aetiology and pathogenesis [23, 24]. Mergental et al. [22] reported tumour size not to be an

important risk factor for post-transplant survival in patients with a non-cirrhotic HCC. Based on these findings, we propose the following treatment strategy for patients with unresectable HCC in a non-cirrhotic liver without well-established risk factors. Patients with extrahepatic spreading or gross vascular involvement should be strictly excluded from liver transplantation. If a biopsy is performed and the presence of a moderately or poorly differentiated tumour with microvascular invasion is noted, caution is needed, especially when the preoperative serum AFP level is high. A large tumour size per se (outside the Milan criteria) should not be seen as a strict contraindication for considering liver transplantation in patients with HCC without well-established risk factors.

This study evaluating a single institution's experience of HCC patients without well-established risk factors is influenced by confounding factors, as the data were acquired retrospectively and might be influenced by patient selection. However, as patients with HCC without underlying cirrhosis are relatively rare, no previous study was able to describe such a large cohort. Although our study was based on a small number of patients ($n = 40$), still we analyzed the largest cohort presented so far. Although the study population is unique, because some of our conclusions are based on a small number of patients (3 patients with local recurrence), experiences from other centres are

needed. However, based on our observations, the absence of well-established risk factors seems to have less impact on final outcome parameters in HCC patients. One can speculate whether the presence of cirrhosis is an independent prognostic parameter or whether the overall assessment of HCC and the recurrence of HCC after treatment with curative intent determine the final outcome in patients with HCC. Therefore, expansion of treatment options by liver transplantation in non-cirrhotic patients is worth reconsidering.

In conclusion, this retrospective analysis of a large cohort of HCC patients without well-established risk factors shows that they were diagnosed at an advanced stage of disease. If surgical resection is performed, good results can be achieved. The higher the preoperative AFP serum level, the worse the outcome. Patients might benefit from major resections, as disease recurrence often appears in the ipsilateral liver lobe instead of the contralateral liver lobe. Although cirrhosis is absent, liver transplantation as a treatment option for HCC patients without well-established risk factors is worth considering and should be further explored.

Disclosure Statement

Nothing to declare.

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