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Role of blood AFP mRNA and tumor grade in the preoperative prognostic evaluation of patients with hepatocellular carcinoma

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Abstract

AIM: To explore the potential prognostic role of preoperative tumor grade and blood AFP mRNA in a cohort of patients with hepatocellular carcinoma (HCC) eligible for radical therapies according to a well-defined treatment algorithm not including nodule size and number as absolute selection criteria.

METHODS: Fifty patients with a diagnosis of HCC were prospectively enrolled in the study. Inclusion criteria were: (1) histological assessment of tumor grade by means of percutaneous biopsies; (2) determination of AFP mRNA status in the blood; (3) patient's eligibility for radical therapies.

RESULTS: At preoperative evaluation, 54% of the study group had a well-differentiated HCC, 42% had AFP mRNA in the blood, 40% had a tumor larger than 5 cm and 56% had more than one nodule. Surgery (resection or liver transplantation) was performed in 29 patients, while 21 had percutaneous ablation procedures. After a median follow-up of 28 mo, 12-, 24-, and 36-mo survival rates were 78%, 58%, and 51%, respectively. Surgical therapy, performance status and three tumor-related variables (AFP mRNA, HCC grade and gross vascular invasion) resulted as significant survival predictors at univariate analysis. Nodule size and number did not perform as significant prognosticators. Multivariate study selected only surgical therapy and a biologically early HCC profile (AFP mRNA negative and well-differentiated tumor without gross vascular invasion) as independent survival variables.

CONCLUSION: The preoperative determination of tumor grade and blood AFP mRNA status may potentially refine the prognostic evaluation of HCC patients and improve the selection process for radical therapies.

Key words: Hepatocellular carcinoma; Prognosis; Treatment policy; Biomarker; Tumor biology

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INTRODUCTION

Liver transplantation (LT), hepatic resection and percutaneous treatments[1-3] are commonly considered as potential radical therapies for patients with hepatocellular carcinoma (HCC). Macroscopic tumor characteristics detected by imaging studies (nodule size and number) undoubtedly identify a subgroup of HCC patients at an early stage of hepatic disease (single nodule <5 cm, <3 nodules, and <3 cm) able to achieve long-term survival figures when radically treated[4,5]. Large pathologic retrospective studies[6-9], however, have shown that these macro-morphological features are indirect markers of specific tumor histological characteristics such as grade of differentiation and microscopic vascular invasion which have therefore the potential to refine the HCC prognostic assessment[10-12]. On the other hand, many molecular biological factors have been studied and related to the HCC biological aggressiveness[13]. However, preoperatively detectable biomarkers (histological and/or hematic) have not yet been introduced in the

routine HCC prognostic evaluation. In our experience, the preoperative determination of the HCC grade by means of percutaneous biopsy performed as a valid tool in the selection of unresectable HCC patients for transplantation[14]. Moreover, we retrospectively showed that some patients with morphologically advanced but well-differentiated HCC may benefit of potentially radical therapies[15]. Among the proposed HCC molecular markers, alpha-fetoprotein messenger RNA (AFP mRNA), detected in the blood by reverse-transcription polymerase chain reaction (RT-PCR), has been the most diffusely studied in last years[16-19]. Our preliminary experience with this marker showed its significance and promising correlation with HCC invasiveness parameters[16]. However, the prognostic utility of AFP mRNA when detected before the treatment remains controversial[20-22]. In this prospective study, a group of HCC patients were selected for radical therapies according to a precise treatment schedule. In addition to conventional HCC parameters, their preoperative study also included the determination of tumor grade and AFP mRNA status in the blood. The enrolled patients were prospectively followed in order to identify the main preoperative predictors of survival.

MATERIALS AND METHODS

Patient enrolment

Between April 2001 and October 2002, 50 patients with a diagnosis of HCC were prospectively enrolled in the study. Patients were eligible for inclusion if they met the following criteria: (1) HCC diagnosis histologically confirmed by the examination of liver specimens obtained by percutaneous biopsies. Pathological grading was contextually assessed in all the patients according to Edmonson's classification; (2) patient's informed consent to provide blood samples for AFP mRNA determination; (3) patient's eligibility for radical therapies according to a published schedule[14,23].

Hepatic resection was considered for HCC patients with preserved liver function (Child-Pugh A-B) and a technically resectable liver tumor without extra hepatic metastasis. If resection was not feasible, patients were considered for LT. The following exclusion criteria for LT were established as previously reported[14]: general contraindications to transplant (age, severe extrahepatic diseases, recent malignancies, and compliance), extra hepatic spread or gross vascular invasion (preoperatively evident or suspected), poorly-differentiated HCC (G3) at pre-OLT percutaneous biopsy. Size and number of nodules were not considered as absolute selection criteria. Patients not fulfilling the criteria for any surgical procedure were assessed for other loco-regional treatments such as percutaneous radiofrequency (RF) or ethanol injection (PEI), and transarterial chemoembolization (TACE).

After discharge, the patients were followed up regularly according to the main treatment received. To detect tumor recurrence after surgery, patients received clinical and laboratory assessment every month, and US and CT every 3rd and 6th mo, respectively. When HCC recurrence was noted, patients were further treated with the most appropriate procedure according to our treatment schedule. After PEI, RF or TACE, dynamic CT examinations were performed to determine therapy efficacy and, when residual staining was noted, these procedures were repeated and the outcome was examined. None of the enrolled patients were lost during follow-up or died within 30 d after first observation or treatment. Therefore, all the 50 patients initially entering the study were available for the survival analysis.

Detection of AFP mRNA in nucleated cells

The AFP mRNA status in the blood was determined in each enrolled patient within 3 mo from treatment application. In brief, total RNA was extracted from 7 mL of peripheral blood and converted to complementary DNA. A nested polymerase chain reaction (PCR) protocol was performed as previously reported[16]. Positive results were evidenced as a 282-bp band in agarose gel stained with ethidium-bromide. As a positive control, cDNA obtained from human hepatocyte was used in each run. Negative controls for each step were also always included.

Statistical analysis

All variables were described by statistical characteristics: categorical data were described by frequency and percentage, whereas continuous data by median (range). Comparison between groups was done by using the χ^2 test or the Fisher's exact test for qualitative variables and the logistic regression for quantitative variables. Follow-up length and survival are expressed as median (range). Recruitment of follow-up data was closed on December 31, 2004. Twenty-four preoperative variables were assessed in the univariate survival analysis: age, sex, performance status, presence of cirrhosis, etiology of cirrhosis, clinical evidence of portal hypertension (presence of esophageal varices, splenomegaly with a platelet count <100 000/mm^3, or ascites), Child-Pugh's classification[24], bilirubin, albumin, prothrombin activity, alkaline phosphatase, gamma-glutamyl transpeptidase, creatinin, aspartate, and alanine aminotransferase, AFP, AFP mRNA blood-status, tumor morphology at preoperative imaging study (TNM, bilobarity, number and size of nodules, gross vascular invasion), differentiation degree at preoperative liver biopsy, and type of treatment. For continuous variables, the cut-off level was their median value. Univariate survival analysis was performed including each variable in a Cox regression model and calculating its related likelihood-ratio of χ^2 and *P* value. Survival curves were calculated using the Kaplan-Meier method and compared by means of the log-rank test. All variables significantly influencing survival in the univariate analysis were analyzed together in a Cox's proportional hazard regression model (multivariate analysis) with the aim of studying the independent contribution of each variable in explaining survivorship. The results of the Cox regression were expressed using both the risk ratios with its related confidence interval and the likelihood ratio of χ^2 with its related *P* value. Analyses were performed using the SAS Institute statistical package

(JMP). Differences were considered significant at $P<0.05$.

RESULTS

The main characteristics of the study group are shown in Table 1. At presentation, only 20% of patients had a compromised general condition due to cancer-related symptoms (PST ⩾1), 63% had a Child B-C cirrhosis and 62% had a clinically relevant portal hypertension. As for tumor characteristics, 52% of patients had a T3-T4 HCC; the tumor was larger than 5 cm in 20 patients (40%), while 28 (56%) had more than one nodule; macroscopic vascular invasion was detected in 12 patients (24%) and 27 patients (54%) had a well-differentiated HCC. AFP mRNA was found in the blood of 21 HCC patients (42%). As previously reported[16], the presence of AFP mRNA in blood was significantly related to cholestatic indices, gross vascular invasion, nodule size, and G2-G3 tumors (Table 1). Surgery was performed in 29 patients (58%), including 22 liver resections and 7 OLT, while 21 patients (42%) had percutaneous ablation procedures eventually associated with TACE.

Survival analysis

As at December 31st 2004, the median follow-up for the whole HCC group (50 patients) was 28 months (range 1-45 mo). Overall mortality was 48% (24 cases) and 12-, 24-, and 36-mo survival rates were 78%, 58%, and 51%, respectively (Figure 1A). Five out of the twenty-four variables had predictive prognostic value for survival in the univariate analysis (Table 1): PST, AFP mRNA, gross vascular invasion, histological grade, and surgical therapy.

A first multivariate study including these five variables selected only surgical treatment as independent survival predictor (Table 2, Figure 1B). Taking into account the strict correlation between AFP mRNA, vascular invasion and HCC grade in the study group[16], in a further analysis we combined these three tumor-related variables in a single parameter defined early HCC (AFP mRNA negative and well-differentiated tumor without gross vascular invasion). This second multivariate study selected tumor early biological status as the most important prognostic factor (Table 2 and Figure 1C).

DISCUSSION

Prognostic prediction and therapeutic decision for HCC patients are currently based in most centers on macroscopic tumor characteristics detected by imaging studies (nodule size and number). In the last decade, an empirical rule

Table 1 Preoperative characteristics of the 50 enrolled HCC patients and univariate survival analysis

Variables	Study group (50 patients)	Likelihood-ratio χ^2 (*P* value)
Median age (yr)	62 (22-88)	2.23 (NS)
Sex (M/F)	41/9	1.35 (NS)
Performance status (PST) ⩾1	10	4.06 (.0438)
Cirrhosis	43	1.12 (NS)
Etiology: HCV/HBV/alcohol/other	29/6/5/10	
viral	35	1.7 (NS)
Portal hypertension	31	0.17 (NS)
Child-Pugh score (A/B/C)	16/24/3	2.31 (NS)
Analytical data		
Bilirubin (μmol/L)	20 (7-233)	0.02 (NS)
Serum albumin (g/L)	35 (21-46)	0.49 (NS)
Prothrombin activity (%)	77 (39-109)	0.02 (NS)
[1]Alkaline phosphatase (U/L)	113 (42-395)	1.33 (NS)
[1]GGT (U/L)	64 (21-864)	3.25 (NS)
Creatinin (μmol/L)	83 (63-166)	1.47 (NS)
AST (U/L)	95 (20-492)	0.48 (NS)
ALT (U/L)	78 (12-469)	0.19 (NS)
Median AFP level (ng/L)	17 (4-1 400)	0.69 (NS)
Positive AFP mRNA	21	5.96 (0.0146)
TNM T3/T4	26	2.8 (NS)
Bilobar	7	2.38 (NS)
Number of nodules (1/2 or 3/>3)	22/23/5	
Multinodular	28	0.99 (NS)
[1]Median nodule size (cm)	4 (1-18)	2.76 (NS)
Nodule >5 cm	20	1.62 (NS)
[1]Gross vascular invasion	12	4.42 (0.0354)
[1,2]Histological grade (I/II/III)	27/16/7	
II-III	23	4.10 (0.0490)
Type of treatment		
Resection/LT/loco-regional therapies	22/7/21	
Surgery	29	16.40 (0.0001)

HCV: hepatitis C virus; HBV: hepatitis B virus; AFP: alpha-fetoprotein; TNM: tumor node metastasis classification; LT: liver transplantation.
[1]Variables significantly related to the presence of AFP mRNA in blood. [2]I: well; II: moderately; and III: poorly differentiated HCC.

Table 2 Multivariate survival analysis in the 50 enrolled HCC patients. Only variables significantly influencing survival at univariate analysis were introduced in the Cox proportional hazard model

Variables	RR (95%CI)	Likelihood-ratio χ^2	*P*
Assessing single variables			
Surgery	2.72 (1.59-5.03)	14.07	0.0002
AFP mRNA	2.00 (0.90-4.82)	2.83	NS
Moderately or poorly differentiated grade	1.20 (0.55-2.41)	0.23	NS
Gross vascular invasion	0.86 (0.48-1.59)	0.24	NS
Performance status >1	1.21(0.64-2.46)	0.31	NS
Combining tumor related variables			
Surgery	5.84 (2.23-17.32)	13.23	0.0003
[1]Early HCC	5.84 (2.23-17.32)	13.60	0.0002
Performance status >1	1.18 (0.41-3.09)	0.11	NS

RR: risk ratio; CI: confidence interval.

[1]AFP mRNA negative and well-differentiated HCC without gross vascular invasion.

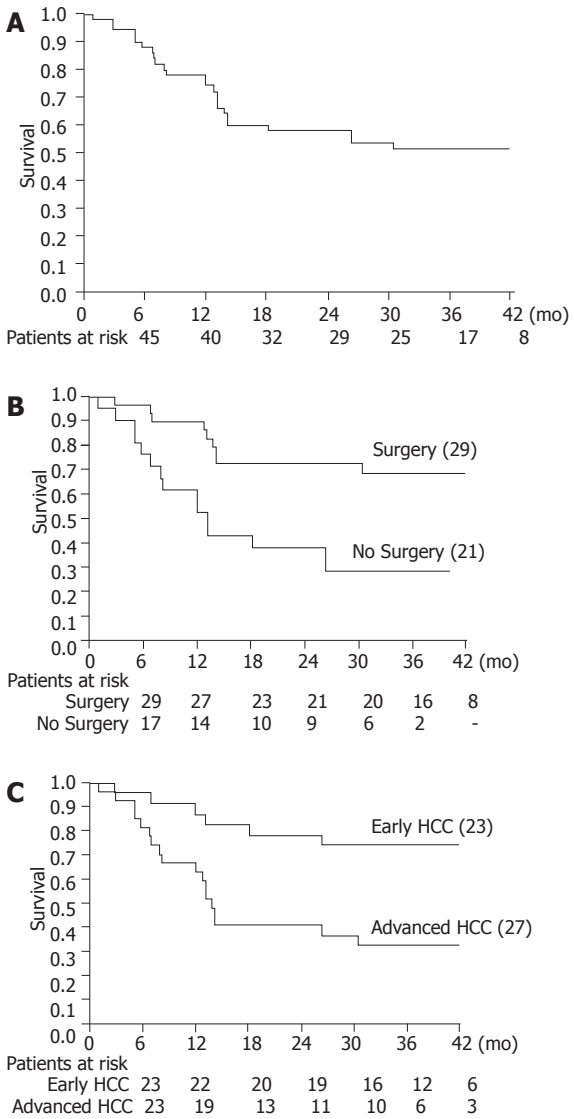

Figure 1 Subject **A:** Overall probability of survival of the 50 enrolled HCC patients; **B:** Overall probability of survival of the 50 enrolled HCC patients, dividing them in the two main treatment groups (surgery *vs* no surgery). Log rank test = 0.0015; **C:** Overall probability of survival of the 50 enrolled HCC patients, dividing them according to tumor biological status (early *vs* advanced HCC). Log rank test = 0.0042. Early HCC = AFP mRNA negative and well-differentiated HCC without gross vascular invasion.

of single nodule smaller than 5 cm in diameter and of multiple nodules (2 or 3) smaller than 3 cm has been used to define early HCC, reflecting the excellent outcomes achieved after LT[25,26]. Although the use of these strict selection criteria for the treatment has undoubtedly improved survival rates of a subgroup of HCC patients in the previous years[4,5], such a diffuse therapeutic policy has dramatically reduced the use of potentially radical therapies for those HCC patients not meeting the same criteria. In virtue of such a tight selection philosophy, on one hand it has obtained an important reallocation of resources but on the other it has faced the concrete risk that a considerable proportion of HCC patients have been unfairly excluded from radical treatment[6-8]. In this context, the identification of new reliable prognostic factors may play a crucial role in improving the treatment criteria process of HCC patients[27].

This study was designed to identify prospectively the main preoperative HCC predictors of survival with particular reference to the prognostic utility of preoperative AFP mRNA and tumor grade. In order to avoid the bias associated to predefined selection criteria, less restrictive enrollment criteria for radical treatment in terms of both tumor status and liver function were deliberately used. If on one hand such a selection policy may account for the relatively unsatisfactory outcome of the 50 enrolled patients (Figure 1A), on the other it allowed us to develop an effective analysis of predictive factors for survival. Moreover, in spite of the relatively low number of enrolled patients, our study has the advantage of a monocentric, prospective analysis in which a well-defined treatment algorithm was used[14,23].

According to other studies[28,29], tumor biological parameters appeared as the most relevant prognostic factors selecting those patients achieving the best survival outcome when radically treated (Tables 1 and 2). In the present study, macro-morphological parameters failed in defining early HCC, since they showed a marginal impact on survival in our group of patients (Table 1). On the contrary, other tumor characteristics such as AFP mRNA in the blood, histological grade and vascular involvement served as more adequate markers of HCC biological

aggressiveness.

In this context, the independent prognostic power of surgical treatment (Table 2 and Figure 1B) may not be misinterpreted as a therapeutic disadvantage in using other loco-regional options. It is not the aim of the present study, in fact, to make a comparison between treatment options since such an analysis may be correctly performed only in the experimental setting of a randomized clinical trial.

This study, in fact, showed that some HCC biological features such as grading, AFP mRNA status in the blood and gross vascular invasion influence patient prognosis independently from the type of adopted therapy (Table 2). A prognostic stratification of the study group in two main tumor stages according to significantly predictive variables was obtained (Figure 1C). A favorable biological picture of HCC (AFP mRNA negative, well-differentiated and without gross vascular invasion) appeared hierarchically as the most relevant factor in determining patient prognosis. Conversely, liver function parameters, such as Child-Pugh classification, bilirubin and portal pressure, did not reach a significant impact on survival in our small study group suggesting a secondary prognostic role when compared to tumor features. In this view, our study does not claim to provide a new definition of early HCC but it wants to simply and strongly underline the urgent need to readdress the focus of the HCC patients selection process for radical treatment more on tumor biological aggressiveness rather than on size and number of tumor nodules.

Concerning the single HCC parameters selected from survival analysis, tumor grade is a well recognized prognostic factor for HCC patients as showed by many large retrospective pathological analyses[1,6,11]. There are, however, no studies evaluating the prognostic role of preoperative determined HCC grading in a prospective setting.

Previous experiences exploring the prognostic role of preoperative AFP mRNA showed controversial results[30-32]. It is likely that this biologic HCC parameter alone does not reach an adequate prognostic power in highly selected populations undergoing radical therapies. In this view, the present study suggests that in the context of a larger population basis, AFP mRNA may be probably useful in identifying morphologically advanced HCC suitable for radical therapies. The introduction of appropriate molecular methods to quantify AFP mRNA in the blood in perspective will further improve the prognostic power of this promising biomarker.

In conclusion, this study showed that the preoperative determination of tumor grade and blood AFP mRNA may potentially refine the prognostic evaluation of HCC patients. On a purely preliminary basis, these two parameters may probably help the treatment decision process for that group of HCC patients suitable for radical therapies consequently improving the currently used restrictive selection criteria.

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