Journal of Internal Medicine 2005; **257**: 367–373

Tumour staging, morphology and p53 overexpression concur in predicting survival in hepatocellular carcinoma

S. GIANNI, A. CECCHETTO, G. ALTAVILLA, R. RAGAZZI, M. BERTAZZO, M. DE GIORGIO, A. BALDAN, S. FAGIUOLI & F. FARINATI

From the Gastroenterology Unit, Department of Surgical and Gastroenterological Sciences, Padua University School of Medicine, Padova, Italy

Abstract. Gianni S, Cecchetto A, Altavilla G, Ragazzi R, Bertazzo M, De Giorgio M, Baldan A, Fagiuoli S, Farinati F (Padua University School of Medicine, Padova, Italy). Tumour staging, morphology and p53 overexpression concur in predicting survival in hepatocellular carcinoma. *J Intern Med* 2005; **257:** 367–373.

Background/aims. The prognosis of hepatocellular carcinoma (HCC) on cirrhosis is hard to predict as it depends on tumour stage, underlying liver disease, type of treatment and, possibly, biological factors of the tumour itself.

Methods. We prospectively evaluated the survival of 91 consecutive patients with HCC on cirrhosis, diagnosed between January 1998 and December 1999. Clinical features and histological/biological aspects, including histotype, grade, p53 overexpression, cytoproliferation and apoptotic markers were analysed.

Results. Child-Pugh ($P = 0.01$), Okuda ($P < 0.0001$), Cancer of the Liver Italian Program (CLIP) staging ($P < 0.0001$) and type of treatment ($P = 0.0001$) were significantly related to survival. In the Cox model, CLIP staging was included as independent predictor of survival at step 1 ($P < 0.0001$) with Okuda at step 2 ($P = 0.013$). Amongst the biological factors, p53 overexpression and histotype were significantly related with survival ($P = 0.0044$ and 0.017 respectively). When clinical and biological variables were examined together in the Cox model, CLIP and Okuda were confirmed as being statistically related with survival ($P < 0.0001$ and $=0.012$) followed by histotype and p53 overexpression ($P = 0.019$ and 0.02).

Conclusions. CLIP, Okuda, histotype and p53 overexpression are the strongest predictors of survival in this series of patients. These data confirm that staging of the tumour and underlying liver disease are strictly related to prognosis but support the concurrent role of clinical and biological factors in upgrading our capacity of predicting the fate of HCC patients.

Keywords: biological neoplastic markers, hepatocellular carcinoma, prognostic factors.

Introduction

Hepatocellular carcinoma (HCC) is a complex disease, in which prognosis depends on tumour stage, phase of the underlying liver disease, that is present in more than 90% of the cases [1], type of treatment the patient affected is eligible for and biological factors of the tumour itself. Tumour staging is the first problem, as patients are usually classified using the classical Okuda's staging system, which dates back in the 1980s [2] or tumour nodes metastasis (TNM), that is generally used in the surgical or transplantation setting [3, 4]. Even the Child-Pugh system for assessing liver residual function [5] is often useful in assessing to what extent the treatment can be aggressive or not and the patients' prognosis. Together with others, we reported on a system, the Cancer of the Liver Italian Program (CLIP) score [6] that both takes into account clinical stage and biological factors, such as alpha-fetoprotein levels and presence of portal thrombosis and that offers an improved prediction of prognosis. We then reported a single-centre experience that confirmed the previous data, also showing that the CLIP

staging may be useful even in subgroups of patients undergoing the same treatment [7]. Treatment is indeed a most relevant factors and the tumour staging proposed by the group in Barcelona Clinic Liver Cancer (BCLC) [8] points to the need of linking a specific stage of the disease to a specific treatment. However, in recent years it has become clear that biological factors are almost as relevant as tumour staging or liver residual function in conditioning the patient's prognosis. Several biological parameters have been tested in the search of a reliable prognostic factor, starting from alpha-fetoprotein, whose levels proved to be related to HCC patients survival also in our experience [9]. Amongst the many others, cytoproliferation has been showed to be linked to prognosis [10–12] as well as with the risk of HCC development in cirrhosis [13], apoptosis has been investigated as were oncogenes and/or tumour suppressor genes [14, 15]. Very seldom however have the above parameters been tested together and in comparison and association with clinical parameters and with treatment to assess their respective relevance.

With this in mind we wanted to assess the respective prognostic roles of a number of clinical and biological parameters in a consecutive series of patients with histologically confirmed HCC in cirrhosis.

Patients and methods

Ninety-one consecutive patients with HCC on cirrhosis were prospectively enrolled in the study in time period included between January 1998 and December 1999. Due to a local policy, diagnosis was always based on the results of fine needle aspiration biopsy with micro-histology (21-gauge fine needle aspiration biopsy, fixed in buffered formaldehyde and stained with haematoxilin and eosin, H & E) and, in most instances also cytology (May–Grunwald–Giemsa and Papanicolau staining) being obtained. With respect to aetiology, patients were grouped according to the findings of anti-HCV antibodies (III° generation ELISA, plus RIBA III confirmatory assay), HbsAg and anti-HBc antibodies plus HBV-DNA, an accurate history with the patient and the family members for alcohol abuse and immunological and biochemical parameters. The demographic features of the series, in terms of age, male/female ratio, aetiology are depicted in Table 1.

Table 1 Demographics of the patients recruited for the study. Patients with HCV-related liver disease represented the majority of the series, as did males

Mean age	63.4 ± 7.8
Male/female ratio	4.3/1
Aetiology (%)	
HBV	11 (12)
HCV	46 (51)
Alcohol	18 (20)
Mixed viruses	4 (4)
Mixed viruses and alcohol	9 (10)
Others	3 (3)

On the basis of clinical, bio-humoral and instrumental features (ultrasound scanning plus at least one other investigation amongst CT scanning, NMR or lipiodol-mediated chemoembolization plus CT scanning at 20–30 days), the patients were subgrouped according to Child-Pugh [5], Okuda [2], TNM [16] and the recently introduced CLIP (Cancer of the Liver Italian Group) staging system [6]. Therapeutic approach, intended as first-line treatment, was chosen according to standard clinical criteria and was based on orthotopic liver transplantation (Mazzaferro's criteria in patients younger than 60 with no systemic contraindications), surgical resection (single node, <5 cm in Child-Pugh A patients), percutaneous treatments (US-guided alcohol injection or radio-frequency thermal ablation for single node HCC with diameter of <5 cm or up to 3 nodes, each <3 cm), lipiodol-mediated chemo-embolization (with lipiodol-emulsionated epirubicin and gelfoam injection, for multiple HCC or single nodes larger than 5 cm or in positions in the liver difficult to approach by either surgery or percutaneous treatment), hormonal treatment [17–21] or simple best supportive care (data also summarized in Table 2).

On sections obtained from the fixed material, the pathologists studied:

1 Histotype (trabecular versus adenoid and mixed forms);
2 Differentiation (Edmonson's grading, with well-differentiated tumours scoring 1, moderately 2 and undifferentiated 3) [22];
3 p53 protein overexpression, by using immunohistochemistry (ICH) method, considering positive or negative findings without grading expression (anti-p53 Mo-Ab – DO-1, IgG 2a; Immunotech S.A., Marceille, France) (Fig. 1);
4 Cyto-proliferation by determining the percentage of MIB1 Ki67-positive cells [Ki67 Mo-Ab (clone

Table 2 Tumour features in terms of number and size of the nodules, staging according to Child-Pugh, Okuda, TNM and CLIP and type of treatment the patients were subject to

No. nodules	
1	43 (48)
2	12 (13)
3	24 (26)
>3	12 (13)
Size of nodules (cm)	
<3	37 (41)
3–5	33 (36)
>3	21 (23)
Child-Pugh	
A	60 (66)
B	24 (26)
C	7 (8)
Okuda	
1	53 (58)
2	33 (36)
3	5 (6)
TNM	
1	8 (9)
2	39 (43)
3	22 (24)
4	22 (24)
CLIP	
0	23 (25)
1	34 (38)
2	22 (24)
3	5 (6)
4	4 (4)
5	3 (3)
No. treatment	13 (14)
Surgery	5 (6)
Transplantation	3 (3)
PEI/RFA	17 (19)
TACE	40 (44)
Hormonal therapy/chemotherapy	13 (14)

Values are presented as *n* (%). CLIP, Cancer of the Liver Italian Program; PEI/RFA, Percutaneous Ethanol Injection/Radio frequency Thermal Ablation.

MIB, IgG1 mouse; Immunotech S.A.], considering negative sections those with <1% positive counted cells per 10 high power fields;

5 Apoptosis, by determining the percentage of BCl 2 positive cells (bcl2clone 124; Dako, Copenhagen, Denmark);

6 CD44, an adhesion molecule whose mutated form plays a role in cell proliferation [23] by determining the cytoplasmic positivity (CD44 std; Bender Medsystems, Vienna, Austria).

After or during treatment, the patients were followed up in our outpatient clinic, mean follow-up being of 20.2 months (range 1–68). In the very few cases in which the patients were lost to follow-up, their records were traced using the demographic registers of the national social security system. Survival analysis was performed by using the log-rank (Wilcoxon–Breslow) test, Kaplan–Meier survival estimates and the Cox multiple regression analysis with the forward conditional stepwise model. Data were considered as significant with a 2p value of <0.05.

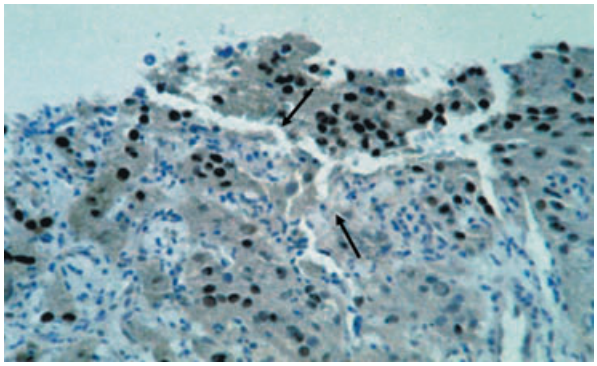

Fig. 1 Large majority of p53-positive nuclei are evident in malignant hepatocytes at the periphery of a bioptic sample from HCC (arrows) (60×).

Results

Patients' features with respect to tumour morphology and biology are summarized in Table 3. Histotype was trabecular in 55% of the cases and adenoid or mixed in 45%, grading 1 was observed in 52.7% with gradings 2 and 3 in 42.9% and 4.4% respectively. Alpha-fetoprotein levels were normal in 36 of 91 patients, slightly increased (<200 ng dL^{-1}) in 37 of 91 and above this level in 18 of 91. Tumours exhibited very low or absent cytoproliferation in 9.9% of the cases, <10% of proliferating neoplastic cells in 18.7% and more than this limit in 71.4%. CD44 positive cells were observed in 70.1% of the cases and BCL2 positive cells in 15.4%. P53 overexpression was observed in 31.9% of the patients.

With respect to survival, only p53 overexpression ($P = 0.0044$) and histotype ($P = 0.017$) were significantly correlated with survival, whilst tumour grading, BCL2 expression, MIB1 and CD44 were not. In the correlation analysis, the following significant correlation were documented:

- Grading with MIB1 ($r = 0.420$, $P = 0.001$) and BCl2 ($r = 0.275$, $P = 0.04$);
- MIB1 with p53 ($r = 0.344$, $P = 0.009$);
- BCl2 with p53 ($r = 0.381$, $P = 0.004$).

Table 3 Tumour morphological features in terms of histotype, grading, and expression of proliferation (MIB1) and apoptosis (BCL2) markers, p53 overexpression and CD44

Tumour morphology	
Histotype	
Trabecular	49 (54)
Adenoid	5 (6)
Mixed	37 (40)
Grading	
Well differentiated	47 (51)
Moderately differentiated	39 (43)
Undifferentiated	5 (6)
Tumour biology	
MIB1	
>10%[a]	64 (70)
<10%[a]	17 (19)
<1%[a]	10 (11)
BCL2	
Negative cells	77 (85)
Positive cells	14 (15)
p53	
Negative	62 (68)
Overexpression	29 (32)
CD44	
Positive cells	64 (70)
Negative cells	27 (30)

Values are presented as *n* (%). [a]Rate of positive cells counted per 10 high power fields.

Table 2 reports the data obtained by staging the patients with the four clinical staging system. Most of the patients were in Okuda I and II (59.4% and 35.1% respectively), TNM staging distribution was the following: 6.6% in stage 1, 44%, 24.1% and 26.3% in stages 2, 3 and 4 respectively. Sixty-seven per cent of the patients were in Child-Pugh A. The large majority of the patients were in group 0, 1 and 2 of CLIP score staging system.

In the single regression analysis, from the clinical point of view Child-Pugh staging ($\chi^2 = 10.6$, $P = 0.01$), Okuda ($\chi^2 = 26.8$, $P = 0.000$) and CLIP staging ($\chi^2 = 37.5$, $P = 0.000$) plus type of treatment ($\chi^2 = 40.1$, $P = 0.0001$) were significantly related to prognosis, whilst aetiology and TNM staging were not ($P = 0.600$ and 0.655 respectively). In particular, median survivals were as follows:

- 43, 13 and 2 months in Okuda 1, 2 and 3 patients respectively;
- 33, 17 and 4 months in Child-Pugh A, B and C respectively;
- 43, 34, 22, 11, 5 and 2 in CLIP scores 0–5;
- 52 for surgery, 48 for percutaneous treatments, 27 for transcatheter arterial chemoembolization (TACE) and 4 months for hormonal treatment and best supportive care (Table 4).

In the Cox forward conditional model, the CLIP staging system was included as single independent predictor of survival at step 1 ($\chi^2 = 15.7$, $P = 0.000$), with Okuda entering the system at step 2 ($\chi^2 = 13.03$, $P = 0.013$). No other factor was required for prediction of survival. When morphological and biological parameters together were taken into consideration, the following results were obtained:

Table 4 Patients' median survival, statistical univariate survival analysis and Cox forward conditional stepwise multivariate analysis

	Score	Median survival (months)	Log rank survival analysis	Cox regression analysis
Child-Pugh	A	33 ± 5	$P = 0.01$	
	B	17 ± 4		
	C	4 ± 1		
Okuda	1	43 ± 23	$P = 0.0001$	$P = 0.013$**
	2	13 ± 5.5		
	3	2 ± 2		
CLIP	0	43 ± 29.1	$P = 0.0001$	$P = 0.0001$*
	1	34 ± 3.7		
	2	22 ± 14.6		
	3	11		
	4	5		
	5	2		
Treatment	Surgery	52	$P = 0.0001$	
	Percutaneous therapy	48		
	TACE	27		
	Hormonal treatment/BSC	4		

BSC, Best Supportive Care. *Single independent predictor of survival at step 1 of analysis.
**Parameter selected at step 2 of the analysis.

1 Histotype was significantly related to prognosis ($\chi^2 = 6.14$, $P = 0.01$), with patients with trabecular type having a median survival of 43 months (95% CI: 22.1–63.8) vs. 15 months (95% CI: 7.4–22.5) in patients with a different histotype, whilst grading was not;
2 Alpha-fetoprotein levels were not significantly related to prognosis, as it was observed for CD44 and apoptosis (BCl2);
3 p53 overexpression was a reliable indicator of prognosis ($\chi^2 = 9.66$, $P = 0.001$) with patients with no overexpression surviving much longer than those with [median survival 43 months (95% CI: 30.3–55.7) vs. 16 months (95% CI: 10.9–21.04)] (Fig. 2).

When the morphological and biochemical parameters were entered in the Cox model, p53 overexpression was entered at step 1 as single independent predictor of survival ($\chi^2 = 9.39$, $P = 0$–002), with histotype being included at step 2 ($\chi^2 = 6.68$, $P = 0.01$).

Finally, clinical, morphological and biological parameters were again examined together by means of the Cox forward conditional stepwise analysis: CLIP staging was entered at step 1 ($P = 0.000$), Okuda at step 2 ($P = 0.012$) histotype at step 3 ($P = 0.019$ and p53 overexpression at step 4 ($P = 0.02$), no other additional variable being included.

Type of treatment was highly correlated to survival in the log rank test, but was not selected at any step in the Cox model, either when it was applied to clinical or to all variable including the biological ones.

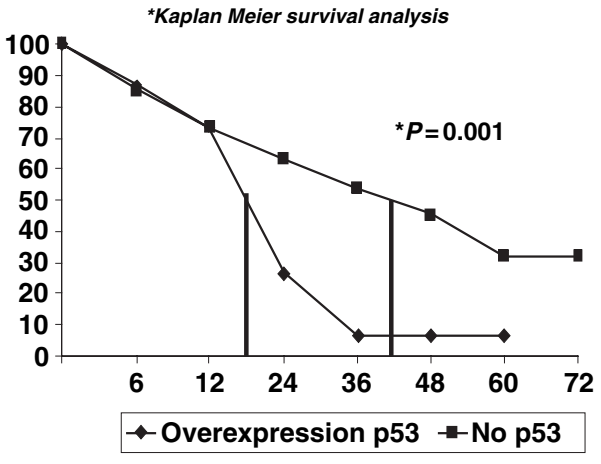

Fig. 2 Prognostic significance of p53 protein overexpression. The survival curves of patients with and without the feature clearly diverge after the first 12 months, with a significant difference in 5-year survival and median survival of 18 and 40 months respectively.

Discussion

Assessment of prognosis in HCC patients is erratic and very often we face long-lasting survival in patients in whom life expectancy should have been limited enough on the basis of clinical staging or, vice versa, short survivals in patients who were actually candidate for having a very good prognosis. This is due to the coincidence of several factors, amongst which:

1 The difference in doubling time between tumour and tumour and between phase and phase in the same tumour [24];
2 The almost invariable association with cirrhosis [25], that is in turn characterized by a life expectancy in itself because of all the potential complications that sometimes are not related to the presence of HCC at all;
3 The difficulty in adequately assessing prognosis with the available clinical staging systems, particularly with TNM staging [16];
4 The varying efficiency of the therapeutic procedures and the high risk of disease relapse [26, 27].

In the last few years several data have pointed to the relevance of tumour biology in assessing the patients' prognosis. AFP has been included in what is apparently by now one of the most efficient staging systems [6] and several factors, such as thrombosis, vascular invasion, severity of the underlying cirrhosis, morphology of the tumour have been used to establish the probability of survival. The present study has the advantage of having assessed prospectively the respective roles of clinical and biological factors in assessing the prognosis of a cohort of consecutive HCC patients referred to our Unit. The results obtained are shown below.

Child-Pugh, Okuda and CLIP staging are all related to prognosis, CLIP staging being also in this series, as it was in a previous one we recently published [7] the most accurate predictor of survival, having the advantage of stratifying the patients in more than three subgroups (actually five) all of which characterized by a clearly different median survival [28].

Tumour H & E morphology is also a relevant prognostic factor, particularly with respect to the

histotype that, when trabecular, characterizes patients with longer life expectancy. This result is not in total agreement with all previous data in the literature [29], but recent data seem to confirm our findings [30].

p53 overexpression is a most relevant biological factor, with patients not showing this feature surviving almost three times longer than patients with this biological change that in the large majority of cases indicate the mutation of this tumour suppressor gene. p53 gene and protein play a central role in cell growth and programmed death. A link between p53 mutation or protein accumulation at immunohistochemistry and higher tumour grade or advanced disease has been demonstrated for several tumours. In HCC, p53 mutation/accumulation influence recurrence after surgery [15] and survival in patients with tumour recurrence [31]. Our data therefore confirm what previously reported, additionally showing that p53 accumulation can be considered as an independent prognostic predictor, irrespective of the type of treatment the patient undergoes to and completes a prognostic model that also includes the clinical features of the patients (interestingly enough with the staging system that includes alpha-fetoprotein) and tumour morphology.

The above observations are confirmed by the fact that when the Cox model was applied to the clinical staging systems, the CLIP score entered the system at step 1, with the highest significance, and Okuda staging was selected at stage 2 and that when it was applied to the biological parameters, p53 overexpression entered the system at step 1, followed by histotype.

Finally, interesting observations are to be derived by the data produced when the Cox model was applied to all the variables together: the Cox model selected four predictors, two from the set of clinical and two from the set of biological/morphological variables and introduced them in the prognostic system in the following order: CLIP, Okuda, histotype and p53 overexpression. These four predictors are necessary and sufficient to predict survival in this series of HCC patients thus confirming the concurrent role of clinical and biological factors in upgrading our capacity of predicting the fate of HCC patients. With the continuous developments in molecular and cytogenetic techniques, in genomics and proteomics we should see more and more new biological prognostic markers that will be tested in the clinical scenario. Prospective studies will be essential in defining their respective roles, and in deciding which of them will be used, in combination with clinical and pathological parameters, in assessing HCC patients' prognosis.

Conflict of interest statement

No conflict of interest was declared.

Acknowledgement

Supported in part by MIUR (Italian Ministry of University and Scientific Research) (ex 40%).

References

1 El-Serag HB. Epidemiology of hepatocellular carcinoma. *Clin Liver Dis* 2001; **5:** 87–107.
2 Okuda K, Ohtsuki T, Obata H *et al.* Natural history of HCC and prognosis in relation to treatment. Study of 850 patients. *Cancer* 1985; **56:** 918–28.
3 Lau H, Fan ST, Ng IO, Wong J. Long term prognosis after hepatectomy for hepatocellular carcinoma: a survival analysis of 204 consecutive patients. *Cancer* 1998; **83:** 2302–11.
4 Herrero JI, Sangro B, Quiroga J *et al.* Influence of tumor characteristics on the outcome of liver transplantation among patients with liver cirrhosis and hepatocellular carcinoma. *Liver Transpl* 2001; **7:** 631–6.
5 Llad L, Virgili J, Figueras J *et al.* A prognostic index of the survival of patients with unresectable hepatocellular carcinoma after transcatheter arterial chemoembolization. *Cancer* 2000; **88:** 50–7.
6 CLIP Group. Prospective validation of the CLIP score: a new prognostic system for patients with cirrhosis and hepatocellular carcinoma. The Cancer of the Liver Italian Program (CLIP) Investigators. *Hepatology* 2000; **31:** 840–5.
7 Farinati F, Rinaldi M, Gianni S, Naccarato R. How should patients with hepatocellular carcinoma be staged? Validation of a new prognostic system. *Cancer* 2000; **89:** 2266–73.
8 Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19:** 329–38.
9 Farinati F, Gianni S. Surveillance for hepatocellular carcinoma in cirrhosis: is it cost-effective? *Eur J Cancer Prev* 2001; **10:** 111–5.
10 Puisieux A, Ozturk M. p53 and hepatocellular carcinoma. *Pathol Biol* 1997; **45:** 864–70.
11 Itoh H, Shiro T, Seki T *et al.* Relationship between p53 overexpression and the proliferative activity in hepatocellular carcinoma. *Int J Mol Med* 2000; **6:** 137–142.
12 Ito Y, Matsuura N, Sakon M *et al.* Both cell proliferation and apoptosis significantly predict shortened disease-free survival in hepatocellular carcinoma. *Br J Cancer* 1999; **81:** 747–751.
13 Qin LX, Tang ZY. The prognostic molecular markers in hepatocellular carcinoma. *World J Gastroenterol* 2002; **8:** 385–392.

14 Ito Y, Matsuura N, Sakon M *et al*. Both cell proliferation and apoptosis significantly predict shortened disease-free survival in hepatocellular carcinoma. *Br J Cancer* 1999; **81:** 747–51.
15 Tang ZY, Qin LX, Wang XM *et al*. Alterations of oncogenes, tumor suppressor genes and growth factors in hepatocellular carcinoma: with relation to tumor size and invasiveness. *Chin Med J* 1998; **111:** 313–8.
16 Ramsey WH, Wu GY. Hepatocellular carcinoma: update on diagnosis and treatment. *Dig Dis* 1995; **13:** 81–91.
17 Mazzaferro V, Regalia E, Doci R *et al*. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334:** 693–9.
18 Livraghi T. Role of percutaneous ethanol injection in the treatment of hepatocellular carcinoma. *Dig Dis* 2001; **19:** 292–300.
19 Bruix J, Llovet JM, Castells A *et al*. Transarterial embolization versus symptomatic treatment in patients with advanced hepatocellular carcinoma: results of a randomized, controlled trial in a single institution. *Hepatology* 1998; **27:** 1578–83.
20 CLIP Group (Cancer of the Liver Italian Program). Tamoxifen in treatment of hepatocellular carcinoma: a randomized controlled trial. *Lancet* 1998; **352:** 17–20.
21 Villa E, Ferretti I, Grottola A *et al*. Hormonal therapy with megestrol in inoperable hepatocellular carcinoma characterized by variant oestrogen receptors. *Br J Cancer* 2001; **84:** 881–5.
22 Lygidakis NJ, Tygat GNJ. Hepatobiliary and pancreatic malignancies. Diagnosis, medical and surgical management. *Ed Micarelli* 1990; **3:** 26–7.
23 Endo K, Terada D. Protein expression of CD44 (standard and variant isoforms) in hepatocellular carcinoma: relationship with tumor grade, clinicopathologic parameters, p53 expression, and patient survival. *J Hepatol* 2000; **32:** 78–84.
24 Ebara M, Hatano R, Fukuda H, Yoshikawa M, Sugiura N, Saisho H. Natural course of small hepatocellular carcinoma with underlying cirrhosis. A study of 30 patients. *Hepatogastroenterology* 1998; **45:** 1214–20.
25 Molmenti EP, Klintmann GB. Liver transplantation in association with hepatocellular carcinoma: an update of the International Tumor Registry. *Liver Transpl* 2002; **8:** 736–48.
26 Llovet JM, Fuster J, Bruix J. Prognosis of hepatocellular carcinoma. *Hepatogastroenterology* 2002; **49:** 7–11.
27 Bruix J, Sherman M, Llovet JM *et al*. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL Conference. *J Hepatol* 2001; **35:** 421–30.
28 Nzeako UC, Goodamn ZD, Ishak KG. Hepatocellular carcinoma in cirrhotic and non-cirrhotic livers. A clinico-pathologic study of 804 North American patients. *Am J Clin Pathol* 1996; **105:** 65–75.
29 Lauwers GY, Terris B, Balis UJ *et al*. Prognostic histologic indicators of curatively resected hepatocellular carcinomas: a multi-institutional analysis of 425 patients with definition of a histologic prognostic index. *Am J Surg Pathol* 2002; **26:** 25–34.
30 Sugo H, Takamori S, Kojima K, Beppu T, Futagawa S. The significance of p53 mutation as an indicator of biologic behaviour of recurrent hepatocellular carcinomas. *Surg Today* 1999; **29:** 849–855.
31 Sheen IS, Jeng KS, Wu Jy. Is p53 gene mutation an indicator of the biological behaviors of recurrence of hepatocellular carcinoma? *World J Gastroenterol* 2003; **9:** 1202–7.

Correspondence: Dr Fabio Farinati, Dipartimento di Scienze Chirurgiche e Gastroenterologiche, Policlinico Universitario, Via Giustiniani 2, 35128 Padova, Italy.
(fax: 0039 049 8760820; e-mail: fabio.farinati@unipd.it).