Pergamon

European Journal of Cancer Vol. 32A, No. 2, pp. 221–225, 1996

Printed in Great Britain
0959–8049/96 $15.00+0.00

0959-8049(95)00542-0

Original Paper

Prognostic Value of Serum Alpha-1-antitrypsin in Hepatocellular Carcinoma

M. Pirisi, C. Fabris, G. Soardo, P. Toniutto, D. Vitulli and E. Bartoli

Cattedra di Medicina Interna, Medical School, University of Udine, Udine, Italy

To evaluate serum alpha-1-antitrypsin (A1AT) as a prognostic factor in hepatocellular carcinoma, we studied 75 consecutive patients (60 male, 15 female, mean age ± SD 63.0 ± 9.3 years) in whom hepatocellular carcinoma developed with pre-existing cirrhosis. Median survival time was 245 days (range 4–1568+). 30 patients had serum A1AT concentration of ≤2.20 g/l (Group A) while 45 (Group B) had alpha-1-antitrypsin > 2.20 g/l. Median survival was 518 days in Group A and 81 days in Group B (Mantel-Cox 20.95, $P < 0.0001$; hazard ratio 0.26, 95% confidence limits 0.15–0.46). By stepwise survival analysis, alpha-1-antitrypsin together with bilirubin, tumour size and blood urea nitrogen were chosen among 17 variables as the only independent predictors of survival. We conclude that measurement of serum A1AT concentration might be useful as an inexpensive, widely available prognostic marker of hepatocellular carcinoma.

Key words: alpha-1-antitrypsin (A1AT), hepatocellular carcinoma, cirrhosis, prognosis
Eur J Cancer, Vol. 32A, No. 2, pp. 221–225, 1996

INTRODUCTION

ACUTE PHASE proteins, such as C-reactive protein and orosomucoid, have been proposed among others as diagnostic markers of hepatocellular carcinoma (HCC) [1, 2]. However, in patients with HCC concurrent with liver cirrhosis, as is the rule in industrialised countries, they do not seem to provide a more sensitive test than alpha-1-fetoprotein [3, 4]. Moreover, since they are expressed as a complex and non-specific reaction to infection and injury, they lack specificity as tumour markers.

In recent years, acute phase proteins have been proposed in clinical research as prognostic rather than diagnostic markers of diseases. For example, the serum concentrations of C-reactive protein and orosomucoid can be combined with albumin and pre-albumin to obtain a Prognostic Inflammatory and Nutritional Index, which is correlated to the risk of death in critically ill adult patients [5]; the concentration of C-reactive protein is correlated with the incidence of coronary events in patients with angina pectoris [6] and predicts poor outcomes in patients with severe unstable angina [7].

Another acute phase reactant, alpha-1-antitrypsin (A1AT), also studied as a diagnostic tool in HCC [8], has been reported to predict survival in patients with HCC [9]. A1AT is appealing as a prognostic marker of HCC because it can be measured simply, reliably and inexpensively; besides, it is commonly included in the routine clinical assessment of suspected HCC, which might occur in the setting of A1AT deficiency [10]. Unfortunately, the efficiency of A1AT as an independent predictor of survival in patients with HCC has not been established in comparison with other variables of widely recognised prognostic significance, such as tumour size, bilirubin and Child–Pugh class [11–15]. Thus, in the present study, we added A1AT determination to widely accepted prognostic indices of survival in HCC to evaluate its contribution to a simple, clinically feasible prognostic score for patients with HCC.

PATIENTS AND METHODS

Baseline data were collected retrospectively, reviewing the records of patients who were first admitted to our institution with a diagnosis of hepatocellular carcinoma and followed up regularly thereafter. Enrolment was between 13 December 1990 and 30 November 1994. We studied a total of 75 consecutive patients (60 male, 15 female, mean age ± S.D. 63.0 ± 9.3, range 33–84) in whom HCC developed with pre-existing liver cirrhosis. Diagnosis of HCC was based on suggestive liver imaging (ultrasound, CT and/or MR scans) in the presence of raised alpha-1-fetoprotein levels (> 400 μg/l) and/or diagnostic histopathological findings. None of the diag-

Correspondence to M. Pirisi.
Received 31 Mar. 1995; accepted 3 Jul. 1995.

noses of HCC resulted from prospective surveillance programmes in patients with cirrhosis, and none of the patients had serum A1AT concentrations below the lower limit of the normal reference range.

The size of the tumour was determined by ultrasonography and/or CT or MR scan. Based on the results of the imaging studies, tumours were divided into the following categories: (a) uninodular ≤5 cm; (b) uninodular > 5 cm; and (c) multifocal or diffuse. 26 patients underwent surgical resection or alcoholisation of the tumour; this treatment was followed by arterial chemo-embolism in 14. 13 patients underwent arterial chemo-embolism alone. 36 patients received only supportive treatment.

During follow-up, 52 patients died. Population registries were the source we used to establish date and cause of death of 14 patients who died outside the hospital. A necropsy was performed on 35 patients who died during a hospital stay. Accordingly, the ultimate causes of death were: progressive liver failure in 13 patients; cachexia in 11; gastro-intestinal bleeding in 9; sepsis in 4; haemoperitoneum in 3; pulmonary thrombo-embolism in 3; mesenteric infarction in 3; acute pancreatitis in 1; stroke in 1; pulmonary oedema in 1. In addition, 1 patient died in a car accident, another died suddenly of a presumed myocardial infarction, and in 1 case no specific cause of death could be traced. At the time the study was terminated, 23 surviving patients had received a minimum follow-up of approximately 8 months (range 236–1568 days; median 766 days). The results presented here include our findings as of 14 March 1995.

Blood chemistry tests were measured as part of the standard profile obtained on admission. A1AT was measured in fresh sera by means of a nephelometric technique (APS Beckman, U.S.A.); reference range for this test is 1.15–2.80 g/l. Alpha-1-fetoprotein was measured by radio-immunoassay (Serono, Italy).

Statistical analysis was performed by means of the BMDP statistical software package [16]. The log-rank test (Mantel–Cox) was used to test the null hypothesis of similarity of survival times among different groups of patients. Survival probabilities were plotted as Kaplan–Meier curves. The hazard ratio (R), which gives the relative event rates in the groups, and 95% confidence interval of R were determined to measure relative survival among groups. Finally, Cox regression analysis with stepwise selection of variables was undertaken in order to ascertain the prognostic value of A1AT relative to that of other relevant factors. The assumption of a linear effect on survival of the continuous variables which were entered in the final model was verified by scattergrams, relating their values to the log-transformed survival time observed in each individual patient who died. Occasional missing values were estimated by regression on all other variables with acceptable values.

RESULTS

An arbitrary cut-off of 2.20 g/l, representing the 95th percentile of a group of 32 healthy blood donors (data not shown), was chosen to divide patients in two groups according to their baseline serum A1AT concentration. Thirty patients had serum A1AT concentration ≤ 2.20 g/l (Group A) while 45 (Group B) had A1AT > 2.20 g/l. A summary of the main characteristics of patients at entry into the study is given in Table 1.

Median survival time was 245 days (range 4–1568+); all but 1 of the censored patients survived longer than this median value. In Group A, median survival was 518 days (lower Brookmeyer-Crowley 95% confidence limits (CL) of median 392 days), whereas it was 81 days in Group B (upper Brookmeyer-Crowley 95% CL of median 190 days). The hazard ratio of patients in Group A in comparison with those in Group B was 0.26 (95% CL 0.15–0.46). The estimated survival curves for the two groups are plotted in Figure 1. The observed difference in survival time between the groups was unlikely to be accidental, as indicated by the log-rank test,

Table 1. Characteristics of the studied population

	Number of patients (%)
Child–Pugh class	
A	35 (46.7)
B	26 (34.7)
C	14 (18.6)
Tumour size	
≤ 5 cm	19 (25.3)
> 5 cm	11 (14.7)
Multinodular/diffuse	45 (60.0)
Treatment	
Surgery or alcoholisation	26 (34.7)
Chemo-embolisation	13 (17.3)
None	36 (48.0)
Ascites	
No	36 (48.0)
Yes	39 (52.0)
Bilirubin (μmol/l)	
≤ 51.3	57 (76.0)
> 51.3	18 (24.0)
Albumin (g/l)	
> 35	15 (20.0)
28–35	41 (54.7)
< 30	19 (25.3)
Alpha-1-fetoprotein (μg/l)	
< 20	27 (36.0)
20–400	24 (32.0)
> 400	24 (32.0)

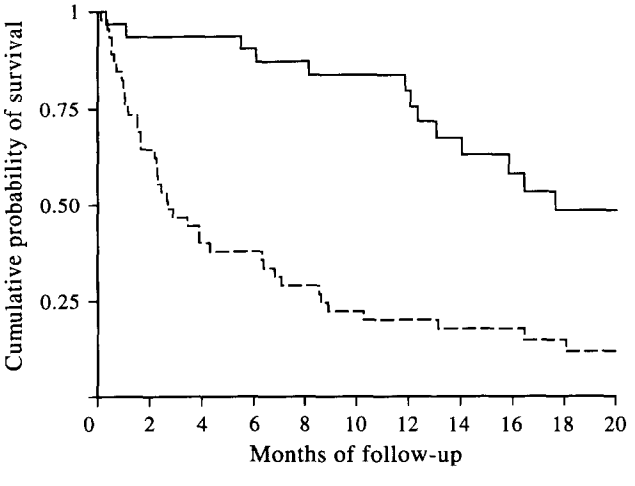

Figure 1. Cumulative survival curves of patients with serum A1AT concentration at baseline (A1AT) ≤ 2.20 g/l (———, $n = 30$) and patients with A1AT > 2.20 g/l (- - - -, $n = 45$).

Table 2. Stratified log-rank test of patients with A1AT ≤ 2.20 g/l (Group A) or > 2.20 g/l (Group B)

Strata*	Mantel–Cox	*P*
Age	20.43	< 0.0001
Sex	23.28	< 0.0001
Treatment	6.18	< 0.05
Bilirubin	22.12	< 0.0001
Alpha-1-fetoprotein	16.42	= 0.0001
Tumour size	13.87	< 0.0005
Child–Pugh class	15.79	= 0.001

* Stratification criteria were the following: age: ≤ 65, > 65 years; sex: female, male; treatment: none, surgery or alcoholisation, chemo-embolism; bilirubin: ≤ 51.3, > 51.3 μmol/l; alpha-1-fetoprotein; < 20, 20–400, > 400 μg/l; tumour size: ≤ 5 cm, > 5 cm or multifocal/diffuse; Child–Pugh class: A, B or C.

which was highly significant (Mantel–Cox 20.95, $P < 0.0001$). This statistical difference persisted even stratifying patients for age at presentation, sex, Child–Pugh class, tumour size, bilirubin, alpha-1-fetoprotein, and type of treatment (Table 2). To generate a Cox regression proportional hazard model, 17 variables were tested on univariate analysis (Table 3). Only those variables with *P* value < 0.10 on univariate analysis were computed on multivariate analysis, using a stepwise approach. Four (tumour size, bilirubin, A1AT and BUN) were found to be independent predictors of survival

Table 3. Survival univariate analysis of selected clinical and blood chemistry variables (log-rank test)

Variable*	Mantel–Cox	*P* value
Age	1.89	> 0.10
Sex	5.44	< 0.05
Treatment	30.04	< 0.0001
Ascites	15.78	= 0.0001
Child–Pugh class	19.64	< 0.0001
Tumour size	8.06	< 0.005
Metastatic disease	8.53	< 0.005
Bilirubin	18.69	< 0.0001
Albumin	8.71	< 0.05
Alkaline phosphatase	11.62	< 0.001
Alpha-1-fetoprotein	9.40	< 0.01
Blood urea nitrogen	3.66	< 0.10
Cholinesterase	6.59	< 0.05
Sodium	3.74	< 0.10
Haematocrit	2.37	> 0.10
γ-glutamyl transferase	18.39	< 0.0001
Prothrombin time (INR)	0.32	> 0.10

* Grouping criteria were the following: age: ≤65, > 65; sex; female, male; treatment: none, surgery or alcoholisation, chemo-embolism; ascites: no, yes; Child–Pugh class: A, B or C; tumour size: ≤ 5 cm, > 5 cm or multifocal/diffuse; metastatic disease: no, yes; bilirubin: ≤ 51.3, > 51.3 μmol/l; albumin: ≥ 35, 28–35; < 28 g/l; alkaline phosphatase: ≤155, >155 U/l alpha-1-fetoprotein: < 20, 20–400, > 400 μg/l; blood urea nitrogen: ≤ 6.4, > 6.4 mmol/l; cholinesterase: ≤ 4000, > 4000 U/l; sodium: ≤ 135, > 135 mmol/l; haematocrit: ≤ 0.38, > 0.38; γ-glutamyl transferase: ≤ 110, > 110 U/l; INR: ≤ 1.23, > 1.23.

(Table 4). Accordingly, a prognostic index (PI) was calculated from the following formula:

$$PI = (6.3027 \times \log A1AT) + (0.7994 \times \text{tumour size}) + (1.8184 \times \log BUN) + (3.5999 \times \log \text{bilirubin}).$$

Two cut-off values of PI were chosen in order to divide the studied population in three groups of approximately equal size. The correspondent Kaplan–Meier curves are plotted in Figure 2. They show the different cumulative survival probabilities of patients with low, intermediate and high PI value.

DISCUSSION

A1AT is a glycoprotein with a molecular weight of 52 kD produced mainly by the liver [17]. Severe, chronic liver disease results because of intracellular accumulation, in the rough endoplasmic reticulum, of variant A1AT, which causes low serum concentrations [18]. Physiologically, A1AT is released like other acute phase reactants via the liberation of cytokines (particularly interleukin-6, interleukin-1 and tumour necrosis factor) [19]. It has been reported that, in patients with cancer, elevated circulating interleukin-6 levels are associated with an acute phase response, which accompanies a reduced fixed hepatic protein synthesis [20]. High A1AT concentrations have been previously observed in patients with HCC and have already been proposed as a diagnostic marker in adjunct to alpha-1-fetoprotein in this condition [8].

The present study reports the survival of a cohort of patients with HCC concurrent with cirrhosis, in relation to a variety of baseline variables. Most of the measurements acknowledged to carry an independent prognostic significance in HCC were included in the analysis. Patients with a serum A1AT concentration ≤ 2.20 g/l, i.e. in the lower part of normal range, were associated with a more favourable outcome, with median survival more than six times longer compared with patients with high A1AT levels. We were able to find only one other report on the usefulness of A1AT determination to predict survival in patients with HCC [9]. In this paper, the authors followed the patients until time of death, with 78 patients enrolled in a prospective, multicentric study between 1976 and 1977, and found the prognostic value of A1AT to be independent of sex, age, HBsAg positivity and alpha-1-fetoprotein concentration. Our findings, obtained in a single centre and with the availability of more modern diagnostic imaging techniques, confirm and extend this original observation. In fact, in the present study, the advantage given by a low A1AT concentration was independent of other relevant and recognised prognostic variables, as demonstrated by stratification prior to log-rank test. The independent prognostic value of A1AT was further supported by multivariate survival analysis with stepwise selection from a set of variables obtained by univariate analysis. A1AT was selected in the final model, in which its prognostic weight was greater than the tumour size and blood urea nitrogen concentration and virtually as ominous as bilirubin. By means of a simple score, our studied population were categorised in three subgroups with well defined differences in survival probabilities.

Does the lower concentration of A1AT of most long-term survivors express a genetic or a pathophysiological difference? A1AT phenotypes determining lower serum levels might result in a more efficient modulation of the cell-mediated immune response and therefore in prolonged survival [21].

Table 4. Stepwise multivariate survival analysis. Continuous variables were retained in their full scale after logarithmic transformation due to their skewed distribution

	Log likelihood	Improvement χ^2	*P* value	Global χ^2	*P* value
Bilirubin (μmol/l)	−165.1	27.9	< 0.001	30.3	< 0.001
Alpha-1-antitrypsin (g/l)	−148.4	33.4	< 0.001	64.2	< 0.001
Blood urea nitrogen (mmol/l)	−142.8	11.2	= 0.001	76.4	< 0.001
Tumour size*	−139.7	6.2	< 0.05	80.8	< 0.001

* Tumour size defined as a categorical variable: 0 = tumour size ≤ 5 cm; 1 = tumour size > 5 cm or multifocal/diffuse.

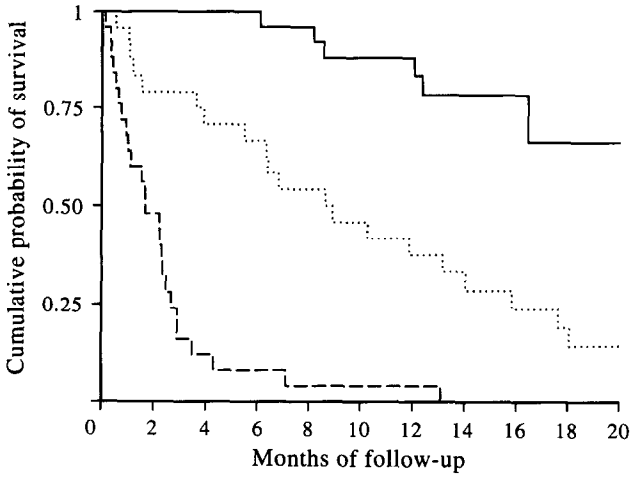

Figure 2. Different cumulative survival probabilities of patients according to a value of prognostic index ≤ 8.93 (———, *n* = 25), > 8.93–≤ 10.69 (. . . . ., *n* = 25), > 10.69 (- - - -, *n* = 25).

Alternatively, high A1AT levels might occur more frequently in patients with the so-called "toxic syndrome" (weight loss ≥ 10%, malaise, anorexia) which is probably cytokine-mediated and is reported to predict a worse survival [11].

The yearly incidence of development of HCC in patients with cirrhosis is approximately 3–4% [22, 23]. In these patients, screening programmes for HCC have been implemented with the hope that HCC might be diagnosed earlier and with a higher resectability rate than in the past. Because of our inclusion criteria, our findings cannot be extended to estimate prognosis in patients diagnosed by screening, in whom therapy might offer better chances. However, the patients in whom HCC is diagnosed by screening are still largely outnumbered by the patients presenting with HCC. Moreover, screening for HCC does not necessarily result in an increased rate of detection of potentially curable tumours [22].

Accuracy in predicting life-expectancy of patients with HCC is of great clinical importance, as the choice of the therapeutic options should be weighed against their respective baseline survival probabilities. So far, we have been unable to identify the type of treatment as an independent predictor of survival in patients with cirrhosis and HCC, in agreement with recent studies by others [11]. Knowing the pretreatment probabilities of survival may allow improved ways of testing the usefulness of all available treatments.

In conclusion, we suggest that the inclusion of measurement of A1AT concentration might improve the accuracy of prognosis estimated in patients with HCC developing in the setting of long-standing cirrhosis.

1. Lee FY, Lee SD, Tsai YT, Wu JC, Lai KH, Lo KJ. Serum C-reactive protein as a serum marker for the diagnosis of hepatocellular carcinoma. *Cancer* 1989, **63**, 1567–1571.
2. Chio LF, Oon CJ. Changes in serum alpha-1-antitrypsin, alpha-1 acid glycoprotein and β2-glycoprotein I in patients with malignant hepatocellular carcinoma. *Cancer* 1979, **43**, 596–604.
3. Fabris C, Pirisi M, Soardo G, *et al.* Value of serum C-reactive protein measurement in the detection of hepatocellular carcinoma superimposing liver cirrhosis. *J Cancer Res Clin Oncol* 1994, **120**, 229–232.
4. Falleti E, Pirisi M, Fabris C, *et al.* Increase of serum α1-acid glycoprotein despite the decline of liver synthetic function in cirrhotics with hepatocellular carcinoma. *Eur J Clin Chem Clin Biochem* 1993, **31**, 407–411.
5. Ingenbleek Y, Carpentier YA. A prognostic inflammatory and nutritional index scoring critically ill patients. *Internatn J Vitamin Nutr Res* 1985, **55**, 91–101.
6. Thompson SG, Kienast J, Pyke SDM, Haverkate F, van de Loo JCW, for the European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *N Engl J Med* 1995, **332**, 635–641.
7. Liuzzo G, Biasucci LM, Gallimore JR, *et al.* The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. *N Engl J Med* 1994, **331**, 417–424.
8. Lee BH, Joo Oj, Ham JS, Lee MH. Serum α1-antitrypsin in patients with hepatocellular carcinoma. *Clin Chim Acta* 1992, **206**, 225–230.
9. Tzonou A, Sparos L, Kalapothaki V, Zavitsanos X, Rebelakos A, Trichopoulos D. α1-antitrypsin and survival in hepatocellular carcinoma. *Br J Cancer* 1990, **61**, 72–73.
10. Propst T, Propst A, Dietze O, Judmaier G, Braunsteiner H, Vogel W. Prevalence of hepatocellular carcinoma in alpha-1-antitrypsin deficiency. *J Hepatol* 1994, **21**, 1006–1011.
11. Calvet X, Bruix J, Gines P, *et al.* Prognostic factors of hepatocellular carcinoma in the West: a multivariate analysis in 206 patients. *Hepatology* 1990, **12**, 753–760.
12. Akashi Y, Koreeda C, Enomoto S, *et al.* Prognosis of unresectable hepatocellular carcinoma: an evaluation based on multivariate analysis of 90 cases. *Hepatology* 1991, **14**, 262–268.
13. Barbara L, Benzi G, Gaiani S, *et al.* Natural history of small untreated hepatocellular carcinoma in cirrhosis: a multivariate analysis of prognostic factors of tumor growth rate and patient survival. *Hepatology* 1992, **16**, 132–137.
14. Rosellini SR, Arienti V, Nanni O, *et al.* Hepatocellular carcinoma: prognostic factors and survival analysis in 135 Italian patients. *J Hepatol* 1992, **16**, 66–72.
15. Haratake J, Takeda S, Kasai T, Nakano S, Tokui N. Predictable factors for estimating prognosis of patients after resection of hepatocellular carcinoma. *Cancer* 1993, **72**, 1178–1183.
16. Dixon WJ, ed. *BMDP Statistical Software Manual.* Berkeley, University of California Press, 1992.
17. Brantly M, Nukiwa T, Crystal RG. Molecular basis of alpha-1-antitrypsin deficiency. *Am J Med* 1988, **84** (Suppl. 6A), 13–31.
18. Perlmutter DH. The cellular basis for liver injury in α1-antitrypsin deficiency. *Hepatology* 1991, **13**, 172–185.
19. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J* 1990, **265**, 621–636.
20. Fearon KCH, McMillan DC, Preston T, Winstanley FP, Cruickshank AM, Shenkin A. Elevated circulating interleukin-6 is associated with an acute-phase response but reduced fixed hepatic

protein synthesis in patients with cancer. *Ann Surg* 1991, **213**, 26–31.
21. Breit SN, Luckhurst E, Penny R. The effect of A1AT on the proliferative response of human peripheral blood lymphocytes. *J Immunol* 1983, **130**, 681–686.
22. Colombo M, De Franchis R, Del Ninno E, *et al.* Hepatocellular carcinoma in Italian patients with cirrhosis. *N Engl J Med* 1991, **325**, 675–680.
23. Ikeda K, Saitoh S, Koida I, *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 1993, **18**, 47–53.