Pergamon

European Journal of Cancer, Vol. 34, No. 8, pp. 1198–1203, 1998

Printed in Great Britain
0959-8049/98 $19.00+0.00

PII: S0959-8049(98)00056-2

Original Paper

Clinical Evaluation of Autoantibodies to p53 Protein in Patients with Chronic Liver Disease and Hepatocellular Carcinoma

J. Raedle,[1] G. Oremek,[1] M. Truschnowitsch,[1] M. Lorenz,[2] W.K. Roth,[3] W.F. Caspary[1] and S. Zeuzem[1]

[1]Second Department of Internal Medicine; [2]Department of General Surgery, Johann Wolfgang Goethe-University, Theodor-Stern-Kai 7, D-60590 Frankfurt/Main; and [3]Chemotherapeutisches Forschungsinstitut, Georg-Speyer-Haus, Frankfurt/Main, Germany

In hepatocellular carcinoma (HCC) of patients from the Western hemisphere, mutations in the *p53* tumour suppressor gene are present in up to 37% of cases. Conformational change and cellular accumulation can initiate an immune response with generation of circulating autoantibodies to p53 protein. In the present study, we investigated 711 consecutive patients with chronic liver disease to evaluate the sensitivity and specificity of autoantibodies to p53 protein as a serological marker for HCC. Detection of p53 autoantibodies was performed using an enzyme-linked immunosorbent assay with immobilised recombinant p53 protein. Liver cirrhosis was present in 259 patients (36.4%) and a HCC was diagnosed in 75 patients (10.6%). Autoantibodies to p53 protein were detectable in 15 of 377 patients with chronic liver disease (4.0%) and in 10 of 259 patients presenting with liver cirrhosis (3.9%). All 25 p53 autoantibody-positive/HCC-negative patients were carefully investigated and no underlying malignancy was clinically detected, suggesting that elevated p53 antibody levels may not exclusively be detectable in patients with malignant disease. In patients with clinically manifest HCC, p53 autoantibodies were detected in 17 of 75 cases, thus resulting in a sensitivity of 22.7% and a specificity of 96.1%. In contrast, assessment of serum α-fetoprotein (AFP) resulted in a sensitivity and specificity of 69.3 and 91.8% (AFP > 20 ng/ml) and 53.3 and 99.1% (AFP > 100 ng/ml) for the detection of HCC, respectively. The data of the present study reveal that the presence of p53 autoantibodies in patients with chronic liver disease is not completely specific for HCC. Moreover, we obtained no direct evidence that p53 autoantibody formation precedes the clinical diagnosis of HCC. However, serological screening for HCC might be improved by combining AFP and p53 autoantibody assays.

Key words: hepatocellular carcinoma, chronic liver disease, p53 autoantibodies, α-fetoprotein, tumour screening
Eur J Cancer, Vol. 34, No. 8, pp. 1198–1203, 1998

INTRODUCTION

WILD-TYPE p53 protein plays an important role in the control of cell proliferation, cell differentiation and induction of programmed cell death [1]. Recently, mutations in the *p53* tumour suppressor gene have been described as the most common genetic alteration during development and progression of malignancy in a wide range of human cancers [2, 3]. In hepatocellular carcinoma (HCC) of patients from the Western hemisphere, alterations in the *p53* tumour suppressor gene can be detected in 11–37% of cases by direct DNA sequencing [4, 5]. The most predominant alterations are missense mutations in four regions of the most conserved domains of the protein encoded by exons 5–8 [6]. According to the site of the mutation, mutant p53 protein can acquire different characteristics. A common feature of mutant p53

Correspondence to S. Zeuzem.
Received 16 Jul. 1997; revised 5 Jan. 1998; accepted 10 Feb. 1998.

protein as characterised *in vitro* is a conformational alteration leading to a prolonged half-life and cellular accumulation. Conformational change and cellular accumulation, together with subsequent release of mutant and normal p53 protein from transformed cells, may initiate an immune response with generation of circulating autoantibodies to p53 protein in various types of cancer [7–14].

With an estimated world-wide incidence of more than one million cases, HCC is one of the most common human malignancies and causes the death of approximately 250 000 patients per year [15]. Epidemiological data have identified hepatitis B, C and D virus infections, alcohol and various toxic agents (e.g. aflatoxin) as the most important risk factors for HCC development [16, 17]. Moreover, patients with liver cirrhosis are at risk of HCC, irrespective of the aetiology of the underlying liver disease [18]. Since early detection of HCC is crucial for providing appropriate therapy, various programmes for tumour screening have been evaluated [19–23]. However, the detection of small, well differentiated HCCs in patients with liver cirrhosis using real-time ultrasonography and computerised tomography (CT) is often limited [24, 25]. In addition, serum α-fetoprotein (AFP), a commonly used marker for HCC, can be normal or only moderately elevated in patients with small cancer lesions [22, 26], while elevated levels are frequently found in non-malignant liver disease [20, 27]. Preliminary studies have shown that p53 autoantibodies can be detected in some patients with HCC [14, 28] raising the question of whether p53 autoantibodies could be useful as an additional screening parameter. Moreover, there was recent evidence that serological detection of p53 autoantibodies may precede the clinical diagnosis of certain tumours, such as angiosarcoma of the liver and lung cancer and may be useful in identifying patients at high cancer risk [10, 13, 29, 30].

The present study was initiated to determine whether elevated p53 autoantibody titres can be clinically used as a reliable serological marker for the detection of hepatocellular carcinoma in patients with chronic liver disease.

PATIENTS AND METHODS

Blood samples were drawn from 711 consecutive patients (422 men, 289 women; mean age at the time of initial presentation, 48.2 ± 14.7 years; range, 13.8–86.5 years) with chronic liver disease of various aetiology referred to our outpatient clinic between June 1994 and May 1996. The diagnosis of chronic liver disease was confirmed clinically, biochemically, serologically, including tests for mitochondrial, nuclear, smooth muscle and liver kidney microsomal antibodies, serum iron, transferrin, ferritin, ceruloplasmin, α_1-antitrypsin, and hepatitis B, C and D virus markers, by abdominal ultrasound or CT, ultrasound guided liver or fine-needle aspiration biopsy and/or laparotomy as appropriate. According to the underlying liver disease, the study population was classified into two groups with either non-viral or viral liver disease. Overall, in the non-viral group, there were 249 patients, including 13 patients with autoimmune chronic hepatitis (3 male, 10 female), 45 patients with primary biliary cirrhosis (6 male, 39 female), 3 male patients with primary sclerosing cholangitis, 43 patients with inherited chronic liver disorders haemochromatosis (35 male, 6 female) and Wilson's disease (1 male, 1 female), 6 female patients with drug-induced chronic hepatitis, 47 patients with alcoholic liver disease (36 male, 11 female) and 92 patients with chronic liver disease of unknown aetiology (53 male, 39 female). The patients with chronic liver disease of unknown aetiology were summarised within the non-virus-related group, as in at least two-thirds of these patients an increased alcohol consumption was suspected. The group of virus-related chronic liver disease consisted of 462 patients including 374 patients with chronic hepatitis C virus infection (225 male, 149 female) and 88 patients with chronic hepatitis B (60 male, 28 female). 8 of the studied HBsAg-positive patients suffered from hepatitis D virus superinfection. The clinical and biochemical characteristics of the investigated patients are summarised in Table 1.

The diagnosis of liver cirrhosis was histologically confirmed or based upon the presence of portal hypertension, i.e. oesophageal or gastric varices and ascites. In 52 of 75 cases (69.3%) with HCC the diagnosis was histologically proven. No tissue samples were obtained from patients presenting with clinically advanced cancer and HCC typical AFP elevations or imaging findings. An underlying liver cirrhosis was present in 66 of 75 patients with HCC (88.0%). HCC localisation was unifocal in 48 of 75 cases (64.0%) and multifocal with several defined lesions in 27 of 75 cases (36.0%). 8 patients had evidence of extrahepatic metastases (10.7%).

All patients with elevated p53 autoantibody or AFP levels without evidence of manifest HCC were carefully evaluated and underwent a complete clinical check-up to exclude any as yet undetected malignant disorder.

Enzyme-linked immunosorbent assay (ELISA) for p53 autoantibodies

At initial presentation, blood was obtained from all patients and centrifuged within 2 h of collection. Serum was stored at − 20°C. The detection of p53 autoantibodies was performed using an ELISA (Dianova, Hamburg, Germany) with solid-phase recombinant wild-type p53 protein. After incubation, the optical density (OD) was determined spectrophotometrically (SLT-Labinstruments, Salzburg, Austria) at 450 nm. Positive control sera containing defined amounts of p53 autoantibodies were provided by the manufacturer. All samples were assayed in duplicate and results are expressed as p53 autoantibody index [$f_i = (OD_{patient} - OD_{low\ control})/(OD_{high\ control} - OD_{low\ control})$]. Values of $f_i > 0$ were considered positive (p53 +). Serum samples from healthy individuals ($n = 135$) serving as healthy controls showed no p53 + results.

AFP measurement

Quantitative determination of AFP in all patients was performed by an enzyme immunological assay (Boehringer Mannheim, Germany). In this two-step sandwich assay, AFP levels > 20 ng/ml were considered elevated.

Statistical analysis

Data are expressed as mean ± standard deviation (S.D.) and as median values with 25–75% quartile ranges when appropriate. Comparisons of patients with chronic liver disease, liver cirrhosis or HCC were performed using a non-parametric Kruskal–Wallis or Fisher's two-tailed exact test. The *U* test (Wilcoxon–Mann–Whitney test) was used to compare the groups of patients with viral and non-viral liver disease. Relationships between variables were examined by Spearman's rank correlation. All *P* values are two-tailed and $P < 0.05$ was judged to be statistically significant. Moreover, sensitivity, specificity, positive/negative predictive value

Table 1. *Clinical characteristics of* 711 *patients with chronic liver disease*

	Non-viral liver disease ($n = 249$)	Viral liver disease ($n = 462$)	P*	Total ($n = 711$)
Patients (male/female)	137/112	285/177		422/289
Age (years) mean ± S.D.	52.7 ± 14.0	45.8 ± 14.5	< 0.001	48.2 ± 14.7
Liver cirrhosis†‡	75 (30.1%)	184 (39.8%)	< 0.05	259 (36.4%)
HCC†‡	43 (17.3%)	32 (6.9%)	< 0.001	75 (10.6%)
Liver functions§				
Serum ALT (U/l) (normal range < 21 U/l)	22.0 (14.0–37.5)	44.0 (27.0–74.0)	< 0.001	36.0 (19.0–62.0)
Serum AST (U/l) (normal range < 17 U/l)	19.0 (12.0–31.0)	28.0 (19.0–49.3)	< 0.001	25.0 (15.0–44.0)
γ-Glutamyltransferase (U/l) (normal range < 23 U/l)	57.0 (28.0–122.0)	34.0 (17.0–61.0)	< 0.001	38.0 (20.0–77.0)
Bilirubin (mg/dl) (normal range 0.3–1.5 mg/dl)	0.7 (0.5–1.2)	0.7 (0.5–1.0)	< 0.01	0.7 (0.5–1.1)
Prothrombin time (% of control) (normal range 75–100%)	99.0 (84.0–100.0)	100.0 (84.8–100.0)	n.s.	100.0 (84.0–100.0)
Albumin (g/dl)‖ (normal range 3.7–5.4 g/dl)	4.3 (3.9–4.5)	4.3 (4.0–4.6)	n.s.	4.3 (4.0–4.6)
γ-Globulin (g/dl)‖ (normal range 1.2–2.2 g/dl)	1.3 (1.2–1.8)	1.6 (1.3–2.0)	< 0.05	1.6 (1.3–2.0)
Cholinesterase (U/l)‖ (normal range 2300–7400 U/l)	4993.0 (3714.5–6305.3)	5193.0 (3823.0–6280.5)	n.s.	5168.0 (3788.5–6280.5)
AFP (ng/ml)§ (normal range < 20 ng/ml)	5.0 (3.5–7.2)	5.5 (3.9–10.6)	< 0.05	5.2 (3.7–9.5)
AFP > 20 ng/ml†	36 (14.5%)	68 (14.7%)	n.s.	104 (14.6%)
AFP > 100 ng/ml†	26 (10.4%)	20 (4.3%)	< 0.01	46 (6.5%)
p53 autoantibody-positive†**	16 (6.4%)	26 (5.6%)	n.s.	42 (5.9%)
p53 autoantibody-positive/HCC-negative†**	8 (3.2%)	17 (3.7%)	n.s.	25 (3.5%)

AFP, α-fetoprotein; n.s., not significant; HCC, hepatocellular carcinoma; ALT, alanine-aminotransferase; AST, aspartate-aminotransferase. *Statistical differences between patients with non-viral and viral liver disease (U test, Fisher's exact test). †Number of patients (%). ‡Histological and clinical evidence. §Data expressed as median (25–75% quartile ranges). ‖Data not available in all patients (albumin, $n = 185$; γ-globulin, $n = 185$; cholinesterase, $n = 433$). **p53 autoantibody index $f_i = (OD_{patient} - OD_{low\ control})/(OD_{high\ control} - OD_{low\ control})$, where OD is the optical density; $f_i > 0$ was considered positive.

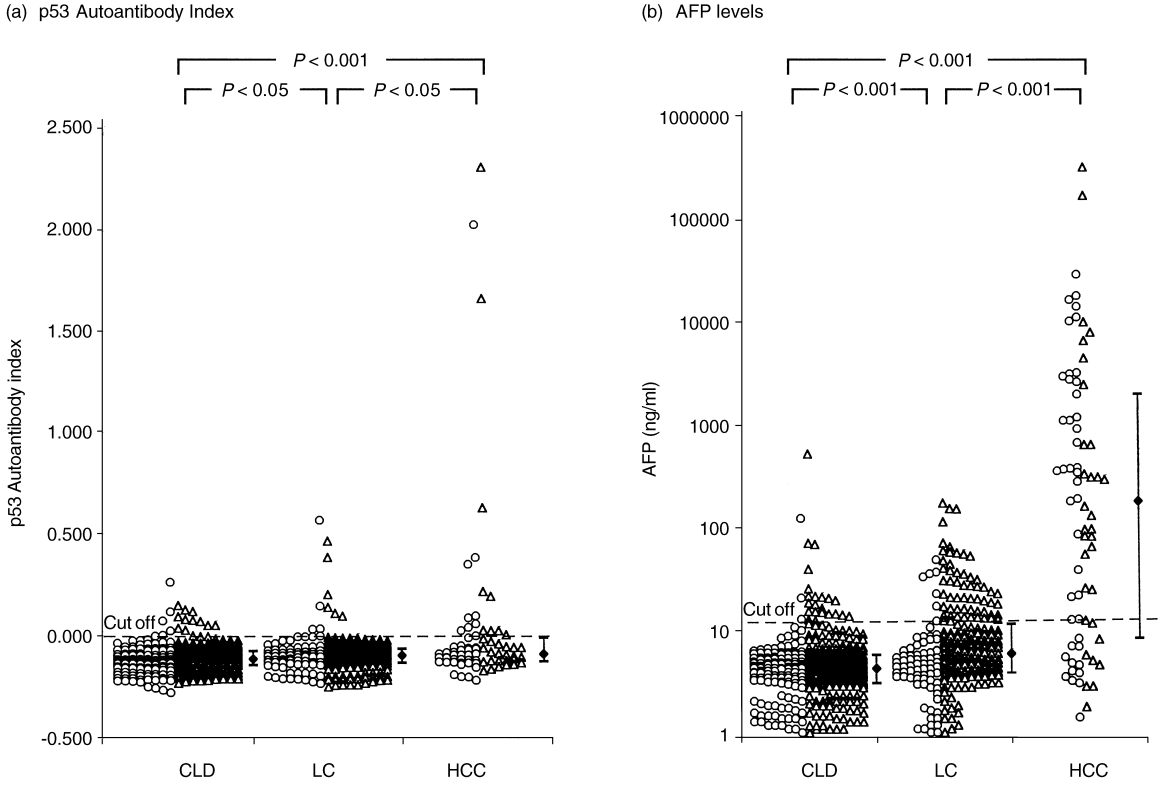

Figure 1. p53 autoantibody index (f_i) (a) and α-fetoprotein (AFP) levels (b) in patients with chronic liver disease (CLD), liver cirrhosis (LC), and hepatocellular carcinoma (HCC). All values of $f_i > 0$ and AFP > 20 ng/ml were considered positive. Median values with 25–75% quartile ranges are indicated (⧫). The non-parametric Kruskal–Wallis test was used for statistical analysis. Patients with non-viral (○) or viral (△) liver disease. p53 autoantibody index: $f_i = (OD_{patient} - OD_{low\ control})/(OD_{high\ control} - OD_{low\ control})$, where OD is the optical density.

(*ppv* +, *npv* −), accuracy, and the absolute and relative Youden indices (Y_S, Y_P) were calculated.

RESULTS

Autoantibodies to p53 protein (p53 +) were detectable in 17 of 75 patients with HCC (22.7%) (Figure 1a). In these patients, AFP levels were elevated above 20 ng/ml in 12 of 17 cases and above 100 ng/ml in 10 of 17 cases. The underlying liver diseases were haemochromatosis ($n = 1$), alcoholic liver disease ($n = 4$), chronic liver disease of unknown aetiology ($n = 3$), hepatitis C virus infection ($n = 5$) and chronic hepatitis B ($n = 4$). No apparent correlation was observed between p53 + status and the aetiology of the chronic liver disease, tumour stage or size.

Furthermore, p53 + levels without evidence of HCC were found in 15 of 377 patients with chronic liver disease without liver cirrhosis (4.0%) and in 10 of 259 patients with liver cirrhosis (3.9%) (Figure 1a). All p53 + /HCC-negative patients (15 men, 10 women; age 42.9 ± 15.5 years) were carefully investigated and no underlying malignant disease was clinically detected. In addition, prospective follow-up of these patients for 105–655 days did not reveal any clinical evidence for malignancy. The underlying chronic liver diseases were autoimmune chronic hepatitis ($n = 2$), primary biliary cirrhosis ($n = 2$), haemochromatosis ($n = 1$), alcoholic liver disease ($n = 3$), hepatitis C virus infection ($n = 10$) and chronic hepatitis B ($n = 7$). All p53 + /HCC-negative patients had a long-standing history of chronic liver disease, but AFP levels were elevated above 20 ng/ml in only 1 case.

Spurious positive readings in ELISA-based assays are known to occur more frequently in patients with hypergammaglobulinaemia and autoimmune disorders. Autoimmune phenomena, such as antimitochondrial antibodies, antinuclear antibodies or rheumatoid factor, were present in 9 of 25 p53 + /HCC-negative patients (36.0%) and in 163 of 430 HCC-negative patients (37.9%) without elevated p53 autoantibodies ($P = 0.51$, not significant). Moreover, there was no relationship between p53 autoantibody status and hypergammaglobulinaemia in the studied patients (data not shown).

Median p53 autoantibody (f_i) levels in patients with chronic liver disease, liver cirrhosis and HCC were − 0.115, −0.098, and −0.092, respectively. Differences among patients with chronic liver disease and HCC or patients with liver cirrhosis and chronic liver disease or HCC proved to be statistically significant ($P < 0.001$ and $P < 0.05$, respectively) (Figure 1a). The sensitivity of the p53 autoantibody assay to detect HCC was 22.7%, with a specificity of 96.1% (Table 2).

In the study population of 711 patients with chronic liver disease, serum AFP levels were raised above 20 ng/ml in 104 of 711 patients (14.6%) and above 100 ng/ml in 46 of 711 patients (6.5%). In 52 of 75 patients with HCC (69.3%), serum AFP levels were raised above 20 ng/ml, but only in 40 of 75 cases (53.3%) above 100 ng/ml, resulting in a sensitivity and specificity of 69.3 and 91.8% (AFP > 20 ng/ml) and 53.3 and 99.1% (AFP > 100 ng/ml) for the detection of HCC, respectively (Table 2). Median AFP levels in patients with chronic liver disease, liver cirrhosis and HCC were 4.6 ng/ml (range 1.0–542.8 ng/ml), 6.3 ng/ml (range 1.0–178.7 ng/ml), and 184.7 ng/ml (range 1.0–326 000.0 ng/ml), respectively (Figure 1b). Differences in serum AFP levels between the three groups were found to be statistically significant ($P < 0.001$ in each case, respectively).

Despite younger age, liver cirrhosis was more frequent in patients with viral liver disease ($P < 0.001$ and $P < 0.05$, respectively) (Table 1). More cases of HCC ($P < 0.001$) and more pronounced elevations of AFP ($P < 0.01$) were observed in the group of patients with non-viral liver disease. No difference was found within both groups for the p53 autoantibody status.

A significant correlation between both tumour markers was excluded by Spearman's rank correlation in all groups of patients. Therefore, circulating p53 autoantibodies and AFP appeared to be independent markers for the detection of HCC in patients with chronic liver disease. According to the data shown in Table 2, AFP > 20 ng/ml and p53 + autoantibody tests had a similar positive predictive value of 40–50% for the detection of HCC by a positive test. The positive predictive value for AFP could be further increased by elevating the cut-off to 100 ng/ml (ppv + = 87%). Furthermore, the accuracy rates of both tests were high (Table 2). Moreover, serological HCC screening was improved by combining measurements of AFP with the detection of p53 autoantibodies at the expense of specificity in patients with chronic liver disease (Table 2).

DISCUSSION

In the present study, p53 autoantibodies and serum AFP levels were measured in 711 consecutive patients with chronic liver disease of different aetiology referred to our hospital. In patients with HCC, elevated p53 autoantibody levels were detected in 17 of 75 cases (22.7%). This prevalence rate is well in accordance with the estimated mutation rate in the *p53* gene, which has been reported to range between 11 and 37% in HCC of patients from the Western hemisphere [4, 5] and the prevalence rate of p53

Table 2. Sensitivity, specificity, positive/negative predictive value (ppv +, npv −), accuracy, relative and absolute Youden indices (Y_S, Y_P) of α-fetoprotein (AFP) and p53 autoantibody index (f_i) for hepatocellular carcinoma (HCC, n = 75) in 711 consecutive patients with chronic liver disease

	Sensitivity* (%)	Specificity† (%)	ppv + (%)	npv − (%)	Accuracy (%)	Y_S‡	Y_P§
AFP > 20 ng/ml	69.3	91.8	50.0	96.2	89.5	0.61	0.46
AFP > 100 ng/ml	53.3	99.1	87.0	94.7	94.2	0.52	0.82
f_i positive‖	22.7	96.1	40.5	91.3	88.3	0.19	0.32
AFP > 20 ng/ml + f_i positive‖	76.0	88.1	42.9	96.9	86.8	0.64	0.40
AFP > 100 ng/ml + f_i positive‖	62.7	95.3	61.0	95.6	91.8	0.58	0.57

*Related to 75 patients with HCC. †Related to 636 patients without HCC. ‡Y_S, absolute Youden index (Y_S = sensitivity + specificity − 1). §Y_P, relative Youden index (Y_P = (ppv +) + (npv −) − 1). ‖p53 autoantibody index $f_i = (OD_{patient} - OD_{low\ control})/(OD_{high\ control} - OD_{low\ control})$, where OD is the optical density; $f_i > 0$ was considered positive.

autoantibodies reported for most types of cancer [8, 10, 13, 29, 31, 32], including one report with p53 + results in 25% of patients suffering from HCC [14]. In this retrospective study of Volkmann and colleagues, only 80 patients with HCC and advanced disease, but no HCC-negative patients with chronic liver disease, were investigated. To determine the specificity of circulating p53 autoantibodies, 711 patients with chronic liver disease of different aetiology with and without liver cirrhosis and HCC were prospectively investigated in the present study.

It is conceivable that liver diseases of different aetiology may affect the rate of mutations in the *p53* tumour suppressor gene [33, 34] and the subsequent immune response in patients with HCC. However, our data revealed no differences in the p53 + status within the different cohorts.

Mutations in the *p53* tumour suppressor gene can cause conformational changes in the p53 protein resulting in a prolonged biological half-life and cellular accumulation. Release of mutant or normal p53 protein is suggested to initiate an immune response with generation of p53 autoantibodies. Previous studies have provided initial evidence that the presence of p53 autoantibodies is highly specific for malignant disorders [7, 8, 13, 14, 28, 35]. In the present study, circulating p53 autoantibodies were undetectable in 135 healthy controls, but were detected in 4% of HCC-negative patients with chronic liver disease independent of the underlying liver disease. The high level of p53 autoantibodies in patients without manifest cancer might be affected by the high percentage of patients with liver cirrhosis and an increased HCC risk, or several patients which suffer from an as yet clinically undetectable cancer. However, extensive clinical investigations revealed no evidence of any malignancy in those patients during follow-up. The proportion of p53 + results in HCC-negative patients with chronic liver disease as observed in the present study is similar to a 5% rate reported in healthy subjects with exposure to vinyl chloride who were screened for angiosarcoma of the liver [30]. In other recent studies, p53 + results were detected in up to 10% of patients with pancreatitis or breast disease without manifest cancer [10, 36]. Nevertheless, a highly significant association between the presence of p53 autoantibodies and malignancy was found in most studies [8, 31, 37].

The presence of p53 autoantibodies may occasionally precede the clinical manifestation of cancer by several months [10, 13, 29, 39]. Mutations in the *p53* tumour suppressor gene can occur early in liver carcinogenesis [6]. It is, therefore, conceivable that the p53 + /HCC-negative patients with chronic liver disease may have an occult HCC. However, during clinical follow-up for up to 655 days, none of these p53 + /HCC-negative patients developed evidence of HCC or any other malignancy. In 10 of 25 p53 + /HCC-negative patients, additional serum samples which had been drawn and frozen prior to study enrolment (range 4–16 months) were available. Nine serum samples were p53 + at that time, giving further clinical evidence that no malignant disease was present in these patients. However, a possibility for prediagnostic potential of p53 autoantibodies remains, since in our study only a small number of prediagnostic sera were tested, with a limited follow-up duration of p53 + /HCC-negative patients.

Moreover, the reason for the presence of p53 autoantibodies in patients without HCC or any other malignancy is unknown. Autoantibodies to p53 recognise both wild-type and mutant conformational and denaturation-resistant epitopes, suggesting that the immunodominant epitopes are outside the mutated domain [9, 11, 12, 38]. Since p53 protein may also be overexpressed in non-tumorous liver cells of patients with liver cirrhosis, a release of p53 protein during cell necrosis/apoptosis occurring in chronic diseases or inflammatory processes might be sufficient to induce an immune response [39]. Furthermore, occasional p53 + readings without manifest cancer may occur more frequently in patients with hypergammaglobulinaemia and autoimmune phenomena [40], but our data revealed no higher incidence of p53 + results in this subset of patients. The ELISA used does not include an immobilised negative control protein as a control for non-specific binding. This may contribute to some false positive results in HCC-negative patients. It is conceivable that the use of a neutral antigen or eucaryotic-expressed recombinant p53 protein may further improve the specificity of the ELISA system.

In the present study, the sensitivity and specificity of serum AFP (> 20 ng/ml) were 69.3% and 91.8%, respectively. Increasing the cut-off level to 100 ng/ml resulted in an improved specificity (99.1%), but reduced the sensitivity from 69.3 to 53.3%. No significant differences were seen in the subgroups of patients with non-viral or viral liver disease (data not shown). In our study, the sensitivity for AFP to detect HCC was higher as compared with two previous studies from Italy and France investigating patients with liver cirrhosis. In 200 Italian patients, Imberti and colleagues reported a sensitivity of 55.3% and a specificity of 82.7% using AFP levels > 20 ng/ml [20]. The data of Pateron and associates revealed a sensitivity and specificity of 50% and 86% for AFP levels > 15 ng/ml and of 21% and 93% for AFP levels > 100 ng/ml in, respectively, 118 French patients [41]. However, these results may be lower due to the study criteria not to include patients with manifest HCC.

As compared with AFP, the sensitivity of p53 autoantibodies for the detection of HCC was low (22.7%), while the specificity reached 96.1%. AFP levels were not related to the presence of p53 autoantibodies. According to our present and previous data and the study of Volkmann and colleagues, a combined assessment of both tumour markers may improve the sensitivity of HCC screening [14, 28], but at the expense of specificity.

Since the majority of patients with HCC have a long-standing history of chronic liver disease and liver cirrhosis, different tumour screening programmes have been evaluated. The data of the present study reveal that the presence of p53 autoantibodies in patients with chronic liver disease is not completely specific for HCC. In contrast to other malignant diseases, we obtained no direct evidence that p53 autoantibody formation precedes the clinical diagnosis of HCC in patients with chronic liver disease [10, 13, 29, 30]. However, serological screening for hepatocellular carcinoma might be improved by combining AFP and p53 autoantibody assays.

1. Harris CC, Hollstein M. Clinical implications of the p53 tumour-suppressor gene. *N Engl J Med* 1995, **329**, 1318–1327.
2. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science* 1991, **253**, 49–53.
3. Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. *Nature* 1991, **351**, 453–456.

4. Challen C, Lunec J, Warren W, Collier J, Bassendine MF. Analysis of the p53 tumor-suppressor gene in hepatocellular carcinomas from Britain. *Hepatology* 1992, **16**, 1362–1366.
5. Volkmann M, Hofmann WJ, Müller M, *et al.* P53 overexpression is frequent in European hepatocellular carcinoma and largely independent of the codon 249 hot spot mutation. *Oncogene* 1994, **9**, 195–204.
6. Harris CC. The 1995 Walter Hubert Lecture—Molecular epidemiology of human cancer: insights from the mutational analysis of the p53 tumour-suppressor gene. *Br J Cancer* 1996, **73**, 261–269.
7. Angelopoulou K, Diamandis EP, Sutherland DJA, Kellen JA, Bunting PS. Prevalence of serum antibodies against the p53 tumor suppressor gene protein in various cancers. *Int J Cancer* 1994, **58**, 480–487.
8. Crawford LV, Pim DC, Bulbrook RD. Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer. *Int J Cancer* 1982, **30**, 403–408.
9. Davidoff AM, Iglehart JD, Marks JR. Immune response to p53 is dependent upon p53/HSP70 complexes in breast cancers. *Proc Natl Acad Sci USA* 1992, **89**, 3439–3442.
10. Green JA, Mudenda B, Jenkins J, *et al.* Serum p53-antibodies: incidence in familial breast cancer. *Eur J Cancer* 1994, **30A**, 580–584.
11. Labrecque S, Naor N, Thomson D, Matlashewski G. Analysis of the anti-p53 antibody response in cancer patients. *Cancer Res* 1993, **53**, 3468–3471.
12. Legros Y, Meyer A, Ory K, Soussi T. Mutations in p53 produce a common conformational effect that can be detected with a panel of monoclonal antibodies directed toward the central part of the p53 protein. *Oncogene* 1994, **9**, 3689–3694.
13. Schlichtholz B, Trédaniel J, Lubin R, Zalcman G, Hirsch A, Soussi T. Analyses of p53 antibodies in sera of patients with lung carcinoma define immunodominant regions in the p53 protein. *Br J Cancer* 1994, **69**, 809–816.
14. Volkmann M, Müller M, Hofmann WJ, *et al.* The humoral immune response to p53 in patients with hepatocellular carcinoma is specific for malignancy and independent of the α-fetoprotein status. *Hepatology* 1993, **18**, 559–565.
15. Munoz N, Bosch FX. Epidemiology of hepatocellular carcinoma. In Okuda K, Ishak KG, eds. *Neoplasms of the Liver*. Tokyo, Springer, 1987, 3–19.
16. 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.
17. Liang TJ, Jeffers LJ, Reddy KR, *et al.* Viral pathogenesis of hepatocellular carcinoma in the United States. *Hepatology* 1993, **18**, 1326–1333.
18. Tsukuma H, Hiyama T, Tanaka S, *et al.* Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993, **328**, 1797–1801.
19. Cottone M, Turri M, Caltagirone M, *et al.* Early detection of hepatocellular carcinoma associated with cirrhosis by ultrasound and α-fetoprotein: a prospective study. *Hepatogastroenterol* 1988, **35**, 101–103.
20. Imberti D, Fornari F, Sbolli G, Buscarini E, Squassante L, Buscarini L. Hepatocellular carcinoma in liver cirrhosis. A prospective study. *Scand J Gastroenterol* 1993, **28**, 540–544.
21. Okuda K. Early recognition of hepatocellular carcinoma. *Hepatology* 1986, **6**, 729–738.
22. Solmi L, Primerano AMM, Gandolfi L. Ultrasound follow-up of patients at risk for hepatocellular carcinoma: results of a prospective study on 360 cases. *Am J Gastroenterol* 1996, **91**, 1189–1194.
23. Zoli M, Magalotti D, Bianchi G, Gueli C, Marchesini G, Pisi E. Efficacy of a surveillance program for early detection of hepatocellular carcinoma. *Cancer* 1996, **78**, 977–985.
24. 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.
25. Ikeda K, Saitoh S, Koida I, *et al.* Imaging diagnosis of small hepatocellular carcinoma. *Hepatology* 1994, **20**, 82–87.
26. Oka H, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of α-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. *Hepatology* 1994, **19**, 61–66.
27. Sato Y, Nakata K, Kato Y, *et al.* Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. *N Engl J Med* 1993, **328**, 1802–1806.
28. Raedle J, Roth WK, Oremek G, Caspary WF, Zeuzem S. α-Fetoprotein and p53 autoantibodies in patients with chronic hepatitis C. *Dig Dis Sci* 1995, **40**, 2587–2594.
29. Lubin R, Zalcman G, Bouchet L, *et al.* Serum p53 antibodies as early markers of lung cancer. *Nature Med* 1995, **1**, 701–702.
30. Trivers GE, Cawley HL, DeBenedetti VMG, *et al.* Anti-p53 antibodies in sera of workers occupationally exposed to vinyl chloride. *J Natl Cancer Inst* 1995, **87**, 1400–1407.
31. Mudenda B, Green JA, Green B, *et al.* The relationship between serum p53 autoantibodies and characteristics of human breast cancer. *Br J Cancer* 1994, **69**, 1115–1119.
32. Peyrat JP, Bonneterre J, Lubin R, Vanlemmens L, Fournier J, Soussi T. Prognostic significance of circulating p53 antibodies in patients undergoing surgery for locoregional breast cancer. *Lancet* 1995, **345**, 621–622.
33. Aguilar F, Harris CC, Sun T, Hollstein M, Cerutti P. Geographic variation of p53 mutational profile in nonmalignant human liver. *Science* 1994, **264**, 1317–1319.
34. Unsal H, Yakicier C, Marçais C, *et al.* Genetic heterogeneity of hepatocellular carcinoma. *Proc Natl Acad Sci USA* 1994, **91**, 822–826.
35. Galle PR, Müller M, Zentgraf H, Volkmann M. Detection of p53-antibodies by immunoblot and ELISA in various human malignancies—a new serological test? In Klapdor R, ed. *Current Tumor Diagnosis: Applications, Clinical Relevance, Research, Trends. Cancer of the Lung–State and Trends in Diagnosis and Therapy*. München, Zuckschwerdt, 1994, 562–564.
36. Raedle J, Oremek G, Welker M, Roth WK, Caspary WF, Zeuzem S. p53 autoantibodies in patients with pancreatitis and pancreatic carcinoma. *Pancreas* 1996, **13**, 241–246.
37. Laurent-Puig P, Lubin R, Semhoun-Ducloux S, *et al.* Antibodies against p53 protein in serum of patients with benign or malignant pancreatic and biliary diseases. *Gut* 1995, **36**, 455–458.
38. Arrowsmith CH, Morin P. New insights into p53 function from structural studies. *Oncogene* 1996, **12**, 1379–1385.
39. Livni N, Eid A, Ilan Y, *et al.* p53 expression in patients with cirrhosis with and without hepatocellular carcinoma. *Cancer* 1995, **75**, 2420–2426.
40. Marxsen J, Schmiegel W, Röder C, *et al.* Detection of the anti-p53 antibody response in malignant and benign pancreatic disease. *Br J Cancer* 1994, **70**, 1031–1034.
41. Pateron D, Ganne N, Trinchet JC, *et al.* Prospective study of screening for hepatocellular carcinoma in Caucasian patients with cirrhosis. *J Hepatol* 1994, **20**, 65–71.

Acknowledgements—The authors thank Dr H. Ackermann, Department of Biostatistics, J.W. Goethe-University, Frankfurt/Main, for statistical advice, Mrs M. Zirker for excellent technical assistance and Professor Dr U. Leuschner, Department of Internal Medicine, J.W. Goethe-University, Frankfurt/Main, for providing the sera of patients with primary biliary cirrhosis.