

Predictive value of liver cell dysplasia for development of hepatocellular carcinoma in patients with non-cirrhotic and cirrhotic chronic viral hepatitis

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Aims: Hepatocellular carcinoma (HCC) frequently develops in patients with chronic viral hepatitis, especially in the cirrhotic stage. We retrospectively studied whether the presence of the putative preneoplastic lesions large liver cell dysplasia (LLCD) and/or small liver cell dysplasia (SLCD) in a needle liver biopsy of these patients are a risk factor for the development of HCC.

Methods and results: The presence of LLCD and SLCD in the needle liver biopsy taken at the initial work-up of 115 patients with chronic hepatitis B or C was assessed retrospectively. LLCD and SLCD were present in the initial biopsy of, respectively, 35 (30%) and

25 patients (22%). During a mean follow-up of 107 months, 16 patients (14%) developed HCC and this occurred significantly more frequently in patients with cirrhosis, age ≥ 55 years, LLCD or SLCD. Cirrhosis and LLCD were independent risk factors for HCC development.

Conclusions: Our findings indicate that the presence of LLCD in a needle liver biopsy of patients with viral-induced chronic liver disease is an independent risk factor for the development of HCC. If these results are confirmed, the presence of LLCD can be used to identify a subgroup of patients at high risk for HCC requiring more intensive screening.

Keywords: chronic viral hepatitis, dysplasia, hepatocellular carcinoma, liver biopsy, risk factor

Introduction

Chronic hepatitis caused by hepatitis B virus (HBV) and hepatitis C virus (HCV) is the major cause of hepatocellular carcinoma (HCC) world-wide.^{1,2} Recent evidence indicates that screening for HCC in patients with chronic viral hepatitis is effective for detecting HCCs at a resectable stage, which leads to a prolonged survival.^{3,4} Using Markov-based decision analysis models, some studies have shown that the cost of screening decreases and the effectiveness improves when screening is performed in a group of patients at high risk for the development of HCC.^{5,6} Thus, identification of additional risk factors for HCC in patients with viral-induced chronic liver disease is important, because a more intensive screening of high-risk patients, e.g. with

computed tomography (CT) scan, could lead to an earlier diagnosis of HCC, which may result in a better survival.

Large liver cell dysplasia (LLCD) and small liver cell dysplasia (SLCD) are morphologically defined lesions which have been proposed to be precursors of HCC.^{7,8} Previous studies have shown that LLCD is a risk factor for HCC in patients with cirrhosis of various aetiology.^{9,10} In the present study, we aimed to determine whether the presence of LLCD and/or SLCD in a needle liver biopsy of patients with viral-induced chronic liver disease are risk factors for the development of HCC.

Patients and methods

PATIENTS

All patients who underwent a needle liver biopsy in the Department of Hepatology in the period between

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January 1985 and January 1993 during their initial work-up and who were diagnosed as having chronic hepatitis B or NANB/C were evaluated for this retrospective study. The diagnosis was based on clinical, serological, histopathological and immunohistochemical data. Patients diagnosed as having chronic hepatitis NANB were considered for inclusion in the study when an anti-HCV test on serum performed in the follow-up period was positive. Patients without follow-up after their initial biopsy and patients with a concomitant liver disease at the time of biopsy were excluded. Evidence for or suspicion of HCC at the time of biopsy also served as an exclusion criterion. The date of the liver biopsy was used as the entry date. The duration of follow-up was the time (measured in months) from the biopsy to either the detection of HCC or the most recent clinical consultation before 1 April 1999 or liver transplantation or death due to other causes than HCC.

The following data at the time of diagnosis were collected from the clinical files: sex, age, smoking habit and use of oral contraceptives for females.

LIVER HISTOPATHOLOGY

The needle liver biopsies were fixed in formalin or Bouin's solution and embedded in paraffin for routine histopathological analysis. Four- μ m thick sections were stained with haematoxylin–eosin (H–E) and sirius red. Immunohistochemical staining was performed using antibodies against hepatitis B core antigen (HBcAg) and hepatitis B surface antigen (HBsAg). The length of the H–E-stained section was measured.

The basic histopathological features of the biopsies were retrospectively evaluated by a pathologist (L.L.) who was unaware of follow-up information of the patients. Cirrhosis was defined according to Knodell *et al.*¹¹ as loss of normal hepatic lobular architecture with fibrous septa separating and surrounding nodules. The presence or absence of cirrhosis was evaluated on the sirius red-stained section. According to Scheuer,¹² chronic hepatitis was classified as inactive or active based on the severity of the periportal/periseptal interface hepatitis (formerly designated as piecemeal necrosis) and the severity of the parenchymal inflammation.

The presence of putative preneoplastic lesions was retrospectively and independently evaluated on the H–E-stained section by two pathologists (L.L. and T.R.) who were unaware of follow-up information. Lesions were only considered present when there was agreement between both observers. LLCD was defined according to Anthony *et al.*⁷ as groups of hepatocytes with nuclear and cellular enlargement, normal

nucleocytoplasmic ratio, nuclear pleomorphism, multinucleation and prominent nucleoli (Figure 1). SLCD was defined according to Watanabe *et al.*⁸ as groups of hepatocytes with decreased cytoplasm, slight nuclear pleomorphism and an increased nucleocytoplasmic ratio, leading to the impression of nuclear crowding (Figure 2). Oncocytic foci were defined according to Gerber *et al.*¹³ as groups of hepatocytes with a dense, finely granular and eosinophilic cytoplasm. Glycogen-storing foci (GSF) were defined according to Altmann¹⁴ as groups of hepatocytes with a clear cytoplasm and a plant-cell-like appearance. The presence of glycogen storage was confirmed on a serial section stained with the periodic acid–Schiff reaction.

STATISTICAL ANALYSIS

Kappa and phi statistics were used to estimate the chance-corrected and chance-independent agreement between the two different observers.^{15,16} Baseline characteristics were compared using the χ^2 test, Fisher's exact test or Wilcoxon test, when appropriate. Quantitative data were expressed as mean \pm SD. The endpoint was the occurrence of HCC. Patients without HCC were censored at the date of the most recent clinical control before 1 April 1999, date of death or date of liver transplantation. The Kaplan–Meier method¹⁷ was used to reflect actual HCC occurrence. Distributions of the occurrence of HCC were compared for each baseline characteristic with the log rank test.¹⁸ A significance level of <0.10 was retained to select the parameters in the multivariate analysis based on the Cox proportional hazards model,¹⁹ using a stepwise-backward procedure with a threshold of 0.05. For all tests, significance was accepted when $P < 0.05$. Statistical analysis was performed using Statview 5.0.1 (SAS Institute, NC, USA) on a Power Macintosh.

Results

One hundred and fifteen patients were included in the study. The biopsies of 56 patients (49%) showed cirrhosis. The study population included 75 men (65%) and 40 women (35%) with a mean age of 50 ± 16 years at the time of biopsy. Seventy-two patients (63%) suffered from a chronic HBV infection and 43 patients (37%) had a chronic HCV infection. Fifty-one patients were smokers, 26 patients were non-smokers and the smoking habits were not known in 38 patients. Use of oral contraceptives was documented in 32 of the 40 female patients and they were taken by six of these 32 patients. Twenty-seven patients (23%) were treated with interferon-alpha (IFN- α) and three

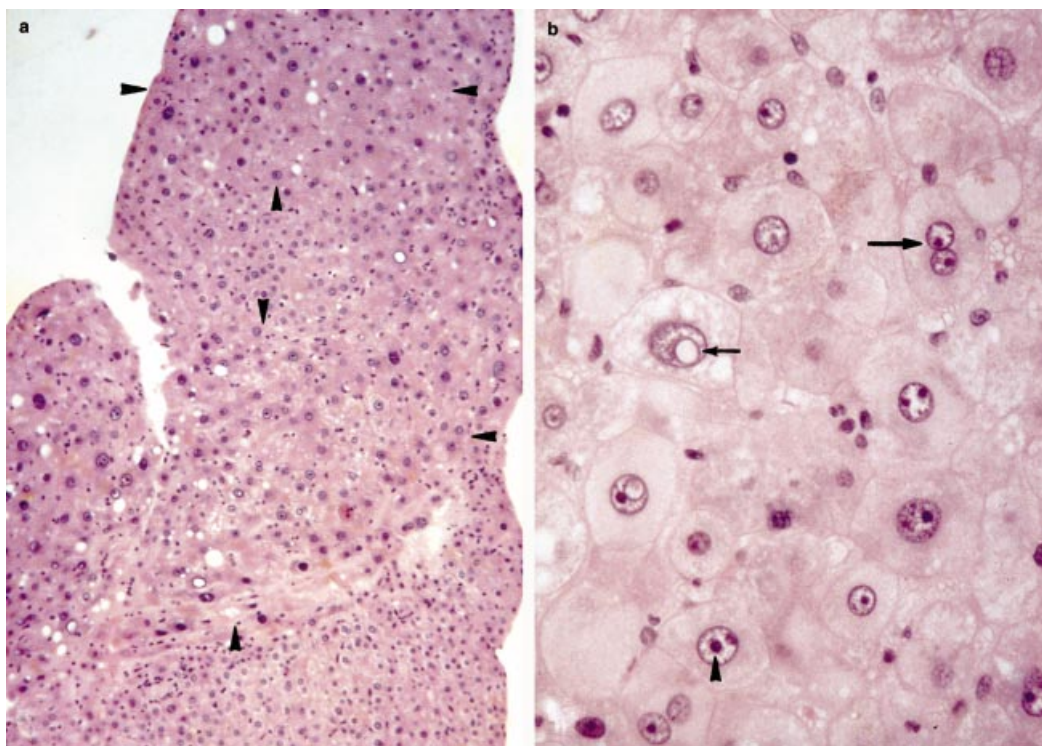


Figure 1. a, Two foci of large liver cell dysplasia (arrowheads) consisting of large hepatocytes with large, pleomorphic nuclei (H-E). b, Large liver cell dysplasia shows prominent nucleoli (arrowhead), nuclear pseudoinclusions (small arrow) and binucleation (large arrow) (H-E).

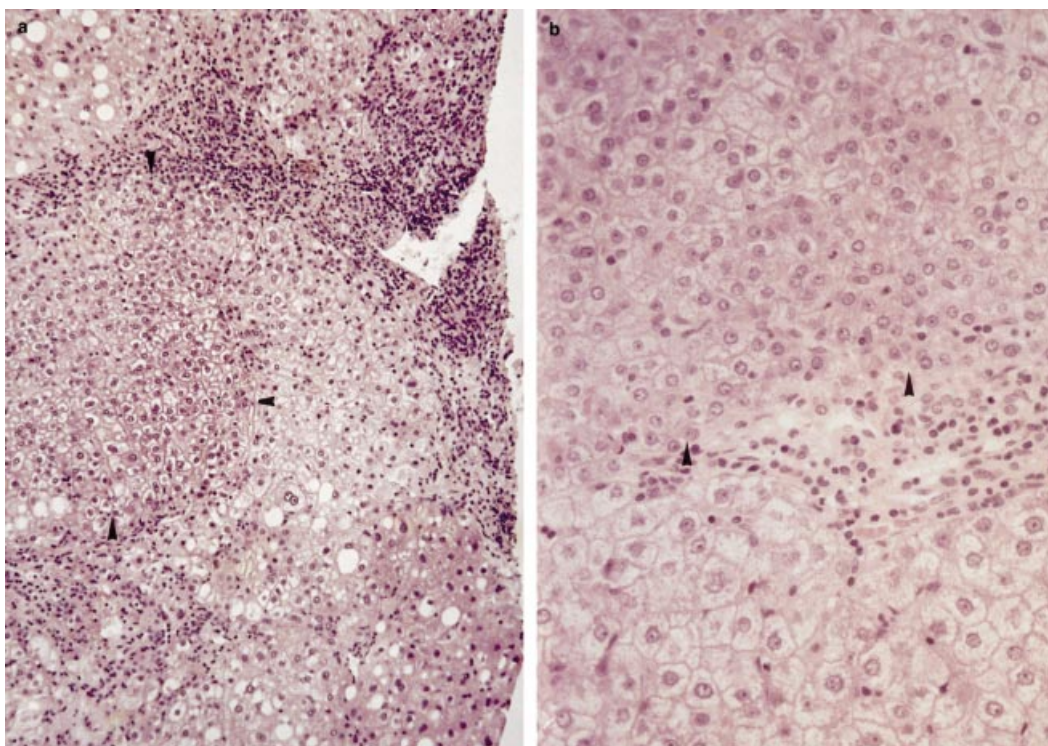


Figure 2. a,b, Small liver cell dysplasia (arrowheads) consists of small hepatocytes with an increased nucleocytoplasmic ratio, giving the impression of nuclear crowding (a, H-E; b, H-E).

patients were diagnosed as abusive alcohol users (>50 g ethanol/day) during follow-up.

During the mean follow-up of 107 ± 46 months, HCC was diagnosed in 16 patients (14%). The diagnosis of HCC was based on histopathological examination of a biopsy in 13 patients and CT and nuclear magnetic resonance imaging (NMR) in three patients. The mean time between the initial biopsy and the diagnosis of HCC was 75 ± 42 months. Of the 13 HCCs diagnosed by histopathological examination, six were classified as well differentiated, five as moderately differentiated and two as poorly differentiated according to the WHO criteria.²⁰ The HCCs had a mean diameter of 41.6 ± 29.6 mm. At the time of this study, 12 of the 16 patients who developed a HCC had died after a mean survival of 18 months. Of the 99 patients who did not develop HCC, 73 were censored at the date of the most recent consultation before 1 April 1999, 22 at the date of death and four at the date of liver transplantation. The mean interval between the initial biopsy and transplantation was 81 ± 33 months. One patient, who had no LLCD or SLCD in the initial biopsy, had no LLCD or SLCD in the explant liver. Of the two patients who had SLCD in the initial biopsy, the explant liver showed SLCD in one patient and SLCD, LLCD and two low-grade dysplastic nodules in the other patient. One patient, who had LLCD in the initial biopsy, had LLCD and a high-grade dysplastic nodule in the liver biopsy.

LLCD was observed in 35 patients (30%) and SLCD in 25 patients (22%). The interobserver agreement was 91% and 81% for LLCD and SLCD, respectively ($\kappa = 0.81$ and $\phi = 0.82$ for LLCD; $\kappa = 0.59$ and $\phi = 0.65$ for SLCD). Sometimes, separate foci of LLCD were observed throughout the parenchyma (Figure 1). LLCD in combination with SLCD was observed in 10 patients (8%). An oncocytic focus was present in four patients and a GSF in two patients. The mean length of the H-E-stained sections was 9 ± 2 mm and did not differ significantly between patients with and without LLCD or SLCD ($P = 0.4419$ for LLCD and $P = 0.2786$ for SLCD). The 22 patients who died did not have a significantly different prevalence of LLCD and SLCD compared with the patients who were still alive at the end of the study ($P = 0.8754$ for LLCD and $P = 0.9006$ for SLCD). The prevalence of LLCD and SLCD was not significantly different between patients treated with IFN- α and non-treated patients ($P = 0.5605$ for LLCD and $P = 0.6428$ for SLCD). Significant correlations between other clinical and histopathological baseline variables and treatment with IFN- α were not present. Chronic hepatitis was active in 65 patients (57%) and the immunohistochemical staining for HbAg was positive in 47 of the 72

patients with chronic hepatitis B (65%). Table 1 shows the values of clinical and histopathological baseline characteristics and their relation to the presence of LLCD and SLCD. The prevalence of SLCD was significantly higher in patients who had an active chronic viral hepatitis. LLCD and SLCD were more frequently present in older patients, in males and in patients with cirrhosis. Patients with SLCD were significantly older and cirrhosis was significantly more frequent compared with patients without SLCD. In the group of patients with chronic viral hepatitis B, the presence of SLCD tended to be correlated with positivity for HbAg. There was no difference in the number of HbAg+ or HBsAg+ hepatocytes in foci of SLCD and LLCD compared with the surrounding parenchyma.

Six of 16 patients who developed HCC (37%) and 19 of 99 patients (19%) who did not develop HCC had SLCD in the initial biopsy. Fourteen of 16 patients (88%) who developed HCC and 21 of 99 patients (21%) who did not develop HCC had LLCD in the initial biopsy. Two of four patients with an oncocytic focus and none of the two patients with a GSF in the initial biopsy developed HCC. Twelve of 16 patients who developed HCC (75%) were cirrhotic at entry. Table 2 shows the results of univariate analysis of the baseline characteristics in relation to the development of HCC. The cumulative probability of developing HCC was significantly higher for patients who were 55 years or older or had LLCD, SLCD or cirrhosis at entry (Table 2, Figures 3 and 4).

The multivariate analysis performed with the four variables selected by univariate analysis revealed that LLCD and cirrhosis were independent risk factors for HCC with a relative risk, respectively, of 16.4 ($P = 0.0003$; 95% confidence interval (CI) 3.621–74.466) and 4.1 ($P = 0.0319$; 95% CI 1.131–15.221). Small cell dysplasia was not a significant independent risk factor after adjustment for cirrhosis and/or large cell dysplasia ($P = 0.5301$; 95% CI 0.479–4.170).

Discussion

The present study strongly suggests that the presence of LLCD in a needle liver biopsy of patients with viral-induced chronic liver disease is an important risk factor for the development of HCC. Several studies in which multiple needle biopsies or large tissue samples from cirrhotic and non-cirrhotic explant or autopsy livers were analysed, revealed that LLCD is widely distributed throughout the parenchyma.^{21–24} Therefore, the risk of sampling errors for LLCD in needle biopsies is small. Our results confirm that there is a good interobserver agreement for LLCD.^{9,10}

Variable	All patients <i>n</i>	Patients with SLCD		Patients with LLCD	
		<i>n</i> (%)	<i>P</i>	<i>n</i> (%)	<i>P</i>
	115	25 (22)		35 (30)	
Age (years)	50 ± 16	57 ± 14	0.0133	55 ± 15	0.0551
Sex			0.2007		0.1768
Male	75	19 (25)		26 (35)	
Female	40	6 (15)		9 (22)	
Cirrhosis			0.0084		0.1087
Yes	56	18 (32)		21 (37)	
No	59	7 (12)		14 (24)	
Virus			0.4401		0.6489
HBV	72	14 (19)		23 (32)	
HCV	43	11 (26)		12 (28)	
Activity			0.0003		0.9292
Yes	65	22 (34)		20 (31)	
No	50	3 (6)		15 (30)	
HBcAg*			0.0735		0.9941
Positive	47	12 (26)		15 (32)	
Negative	25	2 (8)		8 (32)	
Oral contraceptives†			0.5546		0.8847
Yes	6	0 (0)		1 (17)	
No	26	5 (19)		5 (19)	
Smoking‡			0.2090		0.3524
Yes	51	12 (24)		17 (33)	
No	26	3 (12)		6 (23)	

Values in parentheses are row percentages.

*In 72 patients with chronic hepatitis B.

†In 40 female patients; eight missing values.

‡Thirty-two missing values.

The studies of Borzio *et al.*⁹ and Ganne-Carrié *et al.*¹⁰ showed that in patients with cirrhosis of various and unknown aetiology, the relative risk of developing HCC after a mean follow-up of 4 years is, respectively, four to five times greater when the baseline needle liver biopsy showed LLCD. The relative risk in our study was 16, suggesting that LLCD may be a better predictor of HCC in patients with viral-induced chronic liver disease than in patients with cirrhosis of various aetiology. However, the differences in relative risk may also be due to the fact that our study was retrospective or to the fact that the mean duration of follow-up in our study was almost 9 years, which is more than twice longer than in the studies of Borzio *et al.*⁹ and Ganne-Carrié *et al.*¹⁰

A previous study in patients with non-cirrhotic and cirrhotic chronic viral hepatitis due to HCV infection

revealed that the cumulative probability of developing HCC was much higher in patients with LLCD in the baseline needle liver biopsy than in those without.²⁵ However, a recent study did not show a correlation between the presence of LLCD in the needle liver biopsy of patients with HCV-induced cirrhosis and the development of HCC.²⁶ Because both studies were very similar in study design, type and number of patients and duration of follow-up, this difference may be due to differences in the morphological criteria used to define LLCD. While the former study defined LLCD according to Anthony *et al.*,⁷ no definition or example of LLCD was given in the latter study.

Because epidemiological correlations do not prove pathogenic relationships,²⁴ the correlation between the presence of LLCD and development of HCC does not

Table 1. Clinical and histopathological baseline characteristics and their correlation with the presence of large liver cell dysplasia (LLCD) and small liver cell dysplasia (SLCD)

Table 2. Clinical and histopathological baseline characteristics in relation to the development of hepatocellular carcinoma (HCC)

Variable	All patients <i>n</i>	With HCC <i>n</i> (%)	<i>P</i>
	115	16 (14)	
LLCD			< 0.0001
Yes	35	14 (40)	
No	80	2 (3)	
Cirrhosis			0.0012
Yes	56	12 (21)	
No	59	4 (7)	
Age			0.0259
≥ 55 years	52	10 (19)	
< 55 years	63	6 (10)	
SLCD			0.0294
Yes	25	6 (17)	
No	90	10 (11)	
Sex			0.1211
Male	75	13 (17)	
Female	40	3 (8)	
Virus			0.4782
HBV	72	9 (13)	
HCV	43	7 (16)	
Activity			0.7724
Yes	65	9 (14)	
No	50	7 (14)	
HBcAg*			0.1491
Positive	47	5 (11)	
Negative	25	4 (16)	
Smoking†			0.1790
Yes	51	9 (18)	
No	26	2 (8)	
Oral contraceptives‡			0.2985
Yes	6	1 (17)	
No	26	1 (4)	

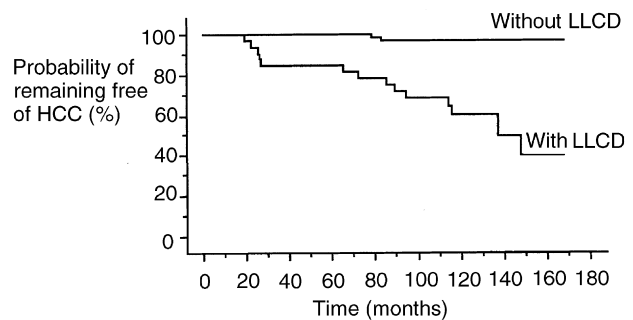
Values in parentheses are row percentages.

*In 72 patients with chronic hepatitis B.

†Thirty-two missing values.

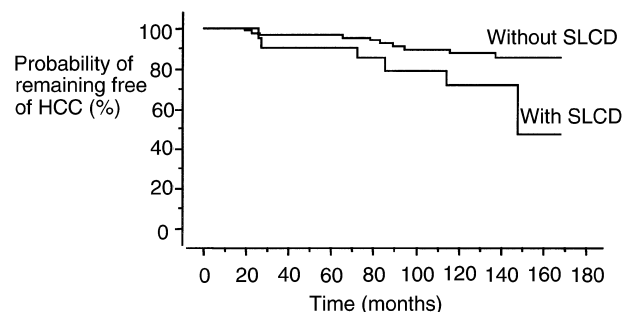
‡In female patients; eight missing values.

imply that LLCD is a direct precursor of HCC. As proposed by Lee *et al.*,²⁷ LLCD might be an 'innocent bystander' which is pathogenically and epidemiologically linked to HCC, but does not represent a direct antecedent of HCC. Therefore, the non-committal term 'large cell change' might be more preferable to LLCD.²⁷

**Figure 3.** Cumulative probability of remaining free of hepatocellular carcinoma (HCC) for patients with and without large liver cell dysplasia (LLCD).

In accordance with Lefkowitz *et al.*,²⁸ patients with HBV infection did not have a significantly different prevalence of LLCD compared with patients with HCV infection. This finding argues against the previously reported suggestion that LLCD is caused by unique HBV-related changes in hepatocytes.^{7,29-31}

To the best of our knowledge, this is the first study which evaluates SLCD in needle liver biopsies of patients with viral-induced chronic liver disease. SLCD was significantly more frequent in patients with cirrhosis (12% in patients without and 32% in patients with cirrhosis) and the cumulative probability of developing HCC was significantly higher in patients with SLCD in the needle liver biopsy. In accordance with Ganne-Carrié *et al.*,¹⁰ the interobserver agreement for SLCD was lower than for LLCD. We found a strong association between active chronic viral hepatitis and the presence of SLCD. In the study of Ganne-Carrié *et al.*,¹⁰ SLCD was associated in 75% of cases with an intense inflammatory infiltrate, which led to the proposition that SLCD is of regenerative nature. The presence of SLCD in conditions usually not associated with development of HCC^{32,33} further supports this hypothesis and it has therefore been proposed to

**Figure 4.** Cumulative probability of remaining free of hepatocellular carcinoma (HCC) for patients with and without small liver cell dysplasia (SLCD).

designate SLCD as 'small cell change'.³⁴ However, in chronic viral hepatitis, the inflammation causes oxidative DNA damage^{35–37} and due to the high proliferation rate which has been measured in SLCD,³⁸ the risk of fixation of genomic alterations is high.³⁹ Moreover, SLCD bears a morphological resemblance to HCC⁸ and a histological continuum between SLCD and HCC has recently been described in a non-cirrhotic alcoholic patient with iron-overload.⁴⁰ Thus, as previously suggested,³⁴ SLCD is most likely heterogeneous and some foci of SLCD might originate from hepatic progenitor cells.^{41–43} While most of the small cell dysplastic foci probably correspond to a regenerative (hyperplastic) phenomenon, some may represent an early step towards HCC.

Foci of altered hepatocytes (FAH) have been found in several animal models of hepatocarcinogenesis⁴⁴ and their preneoplastic nature has been well documented.⁴⁵ Su *et al.* observed FAH in 84 of 111 human cirrhotic explanted and resected livers and the prevalence of FAH was higher in patients with HCC than in those without.²³ In the present study two types of FAH were recognized, i.e. GSF and oncocyctic foci. The prevalence of these foci in the needle biopsies in our study was much lower than in the large tissue samples taken from explanted and resected livers in the study of Su *et al.*,²³ probably because FAH generally do not occur widespread throughout the parenchyma. Interestingly, two of the four patients having an oncocyctic focus in the initial biopsy developed HCC during follow-up.

In patients with HCV-associated cirrhosis, it has been suggested that HCC develops more rapidly when there is a continuous elevation of serum alanine aminotransferase levels, which represents persistent severe inflammation and hepatocyte necrosis.⁴⁶ We observed no correlation between active chronic viral hepatitis and development of HCC, but this is probably related to the fact that the severity of inflammation was assessed at only one point in time.

It is well known that cirrhosis *per se* predisposes to HCC, the relative risk varying according to the aetiology of cirrhosis.² The relative risk of cirrhosis caused by chronic viral infection was 4.1 in the present study and 3.8 in the study of Shibata *et al.*²⁵ In agreement with previous epidemiological studies, HCC occurred more frequently in men and at older age.¹

In accordance with other studies,² we found no evidence of an association between smoking or oral contraceptives and HCC. However, this may be due to the limited number of cases in the present study.

In conclusion, the presence of LLCD in a needle liver biopsy from patients with non-cirrhotic and cirrhotic chronic viral hepatitis is an important independent risk

factor for the development of HCC. Pathologists should look for dysplasia in needle liver biopsies from these patients and report the presence of this lesion to the clinician who is treating the patient. If our results are confirmed, LLCD can be used as one of the criteria for defining a subgroup of patients at high risk for the development of HCC. More intensive screening of this subgroup could lead to an earlier diagnosis of HCC, which may result in a better survival.

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