ORIGINAL ARTICLE

Hepatocarcinogenesis of regenerative and dysplastic nodules in Chinese patients

CME

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Objectives To determine the development rate of hepatocellular carcinoma and survival of patients diagnosed to have regenerative, and low-grade and high-grade dysplastic liver nodules.

Design Retrospective descriptive study.

Setting Acute public hospital, Hong Kong.

Patients Patients with non-malignant liver nodules confirmed by imaging-guided liver biopsy between January 1997 and December 2008.

Main outcome measures Rates of hepatocellular carcinoma development and survival.

Results A total of 147 patients with non-malignant liver nodules were followed up over a median duration of 29 months. The initial histological diagnosis included regenerative nodules (n=74), low-grade dysplastic nodules (n=34), and high-grade dysplastic nodules (n=39). The respective cumulative hepatocellular carcinoma development rate during the first, second, third, and fourth year were 3%, 5%, 9% and 12% for simple regenerative nodules, 29%, 35%, 38% and 44% for low-grade dysplastic nodules, and 38%, 41%, 51% and 51% for high-grade dysplastic nodules. The hepatocellular carcinoma development rate was highest in those with high-grade dysplastic nodules. Multivariate analysis showed that histological dysplastic changes were associated with increased alpha-fetoprotein levels and advanced age, which were both independent predictors of hepatocellular carcinoma development. Histological dysplastic changes, male sex, advanced age, prolonged prothrombin time, and ultrasound appearances were independent predictors of mortality.

Conclusion The presence of dysplastic change in liver nodules increased the risk of hepatocellular carcinoma development and death.

Introduction

Hepatocellular carcinoma (HCC) is one of the major health problems of the world. It is the sixth most frequent cancer in the world and the third most common cause of cancer mortality.[1] Hepatocellular carcinoma occurs mostly in patients with chronic liver disease due to chronic viral hepatitis and alcohol abuse, with hepatitis B virus (HBV) infection being the most common cause of HCC in Hong Kong.[2]

Although HCC is common, its exact pathogenesis is not well understood. One of the popular hypotheses of hepatocarcinogenesis is that it is a 'multistep' process, similar to that described for colorectal cancer.[3] This mechanism envisages that HCC develops from cirrhotic or regenerative nodules (RNs) composed of proliferating hepatocytes. The increase in cell proliferation predisposes them to carcinogenic 'hits', thereby giving rise to cellular atypia (dysplastic foci). These RNs containing cellular or architectural atypia have been referred as adenomatous hyperplasia, macroregenerative nodules, or dysplastic nodules (DNs). With further accumulation of mutational events and aberrant growth, DNs may ultimately transform into HCC.[4] According to this hypothesis, RNs and DNs are intermediate steps in HCC development.

Dysplastic nodules are nodular lesions that differ from the surrounding parenchyma with regard to size and texture. They are usually, but not always, detected in cirrhotic livers.[5] Dysplastic nodules are classified as low-grade (LGDNs) or high-grade (HGDNs) on the basis of cytological and architectural atypia. In general, the hepatocytes in LGDNs

Key words
Carcinoma, hepatocellular; Cell transformation, neoplastic; Liver cirrhosis; Liver neoplasms; Survival rate

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華籍病人中再生結節和增生結節發展形成的肝癌病變

目的 研究患有再生結節、低級別或高級別增生結節的病人，其發展成肝癌病變的速度及存活率。

設計 回顧性描述研究。

安排 香港一所急診公立醫院。

患者 1997年1月至2008年12月期間，影像導航下肝臟活檢確診為良性肝結節的患者。

主要結果測量 發展成肝癌的速度及患者存活率。

結果 147位良性肝結節患者的隨訪期中位數為29個月。經組織學初步確診的再生結節有74例、低級別增生結節34例和高級別增生結節39例。至於第一、二、三及四年的累積發展形成肝癌速度，再生結節病例分別為3%、5%、9%及12%，低級別增生結節病例分別為29%、35%、38%及44%，高級別增生結節病例分別為38%、41%、51%及51%。高級別增生結節患者最快發展成肝癌。多元分析顯示組織學增生病變與高甲胎蛋白水平及高齡有關，而兩者亦是肝癌的獨立預測因子。組織學增生病變、男性、高齡、凝血酶原時間延長和超聲像圖特徵都是死亡的獨立預測因子。

結論 肝結節的增生病變會提高發展成肝癌的速度及死亡風險。

are usually normal in appearance or show only minimal nuclear atypia with a slightly raised nuclear-cytoplasmic ratio. The cytoplasmic features may be similar to the surrounding parenchyma. In contrast, HGDNs show cytological or architectural atypia that approach, but not quite reach, those of HCC. Cytological atypia include: nuclear hyperchromasia, mild nuclear contour irregularities, cytoplasmic basophilia or clear cell change, high nuclear-cytoplasmic ratios (crowding of nuclei), and presence of mitotic figures.[6] A standard for nomenclatures and histological features distinguishing RNs, LGDNs, and HGDNs was proposed by an international working party in 1995.[5]

In the present study, we report the outcomes of our series of histologically diagnosed RNs and DNs. We aimed to confirm the possible malignant potential of DNs and establish the predictive significance of histological, biochemical, and radiological features.

Methods

Patients

This was a retrospective study of patients with imaging-guided liver biopsy of liver nodules performed between January 1997 and December 2008; all specimens were sent to the histopathology laboratory of Tuen Mun Hospital. The patient list (retrieved from the histopathology laboratory database) included all liver specimens processed in their laboratory since 1997. Medical records including patient baseline characteristics; and imaging, biochemical and histological findings were retrieved. Patient inclusion criteria were used: (a) liver nodule detected on imaging; (b) histology confirmed the nodule was non-malignant; (c) alpha-fetoprotein (AFP) level of 200 ng/mL or below; (d) age of 18 years or above; and (e) no history of HCC. Patients with liver nodules without histological confirmation were excluded.

The following data were retrospectively collected from the patient medical records: demographics (age, gender, alcoholic intake history), imaging findings (size, location, ultrasonography [USG] and contrast-enhanced computed tomography [CT] features), and biochemical results in the 3 months prior to biopsy. The latter, which included serum albumin and bilirubin, platelet count, prothrombin time, AFP level, hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg) and antibodies to hepatitis C (HCV Ab) were also recorded. Data were collected on standardised forms by a single investigator. Only one nodule from a single patient was selected as an index nodule and used for study purposes. For those with more than one liver nodule, the nodule with the highest grade of dysplasia was selected as the index nodule. If two or more nodules in the same grade were observed in the same patient, only the largest nodule was utilised.

Histological diagnosis

All biopsy specimens were obtained by percutaneous trucut needle biopsy using an 18-to-20-gauge needle under imaging guidance. Patients were classified into three groups, namely, those with RNs, LGDNs, and HGDNs, according to their histology report. These criteria for classifying liver nodules were based on the consensus of an international working party in 1995.[5] In order to eliminate interobserver variation in grading of dysplasia, all histology slides were reviewed by single experienced hepatopathologist who assigned the grade of dysplasia.

Imaging analysis

All nodules underwent imaging before patients were enrolled, either by USG, contrast-enhanced CT, or both. In our centre, USG was performed with B-mode fundamental and harmonic imaging. Dynamic CT scans were performed in our centre using a single detector helical scanner (PQ6000; Picker, US) before April 2005 and 16-slice scanners (Philips, Brilliance) thereafter. In these studies, 80 mL of non-ionic contrast agent (Iomeron 400; Bracco Atlanta, Germany) were injected intravenously according to our departmental protocol and arterial phase and portal venous

phase scans imaged at 35 seconds and 70 seconds after the injection. The films were reviewed by an independent experienced radiologist who blinded to the histological diagnosis of the liver nodules.

Hepatocellular carcinoma development

In the current study, a patient was diagnosed as having an HCC if he/she fulfilled the criteria proposed by the aforementioned international party.[7] In brief, these criteria were: (1) histological confirmation of HCC; (2) any liver nodules larger than 2 cm in size with a serum AFP level of more than 200 ng/mL; (3) typical imaging features in one imaging modality if the lesion size was more than 2 cm; (4) typical imaging features in two imaging modalities if lesion size was between 1 and 2 cm.

Statistical analysis

Unless otherwise stated, results were presented as medians with ranges, or proportions with percentages. Patients with missing data for an item were eliminated from the analyses involving that particular parameter. To investigate for any differences in background features, laboratory and radiological data among the three groups, baseline data were analysed by Chi squared test and Kruskal-Wallis test, whenever appropriate.

The HCC development time was defined as the period elapsing from the day of the biopsy to the day of diagnosis of HCC. For persons lost to follow-up or not developing an HCC, they were censored at last contact, either at their last out-patient follow-up date or the date of last discharge as an in-patient. Patients were divided into three groups for analysis according to the initial histological findings, ie RNs, LGDNs, and HGDNs group. To elucidate predictors of HCC development, the univariate Cox regression method was used to estimate the cumulative probability of developing an HCC during the follow-up. Risk factors including male gender, HBsAg positivity, HBeAg positivity, HCV Ab positivity, habitual alcohol drinking, Child-Pugh stage >A, being hyper-echoic on USG, being hyper-attenuating on CT, and baseline histology were entered into the model as categorical variables. Age, albumin level, bilirubin level, prothrombin time, and platelet count were entered as continuous variables. To adjust for confounding variables, those with a P value of less than 0.1 were also subjected to multiple regression analysis using the Cox proportional hazard model. We adopted the stepwise backward selection model to select parameters which independently affected HCC development.

For survival analysis, survival time was defined as the period from the date of biopsy to the date of death. Similar to HCC development time analysis, persons lost to follow-up and those who survived were considered as censored since their last contact. Univariate analysis of predictors of death was analysed by univariate Cox regression as mentioned for HCC development analysis. In the second step, a multivariate backward stepwise Cox regression model was used for variables that were significant in the univariate analysis. All data analyses were performed using the Statistical Package for the Social Sciences (Windows version 16.0; SPSS Inc, Chicago [IL], US). A P value of less than 0.05 was set as the level of statistical significance unless otherwise stated.

Results

Patient characteristics

During the years January 1997 to December 2008, there were a total of 987 liver specimens sent to the histopathology laboratory of our hospital, of which 476 were imaging-guided biopsies. After excluding metastatic cancer (n=161), HCC (n=131), haemangioma (n=19), and inflammation and hepatic adenoma (n=18), 147 non-malignant nodules remained. In these 147 nodules, 34 showed histological features of LGDNs and 39 were classified as HGDNs. The remaining 74 nodules did not show any abnormal histological features and were therefore classified as RNs. The clinical characteristics of these 147 patients are shown in Table 1.

Regarding the quality of liver specimens, more than 94% had entailed two or more tissue cores, and 82% were 1 cm or larger in length; their median size was 15 mm (range, 4-24 mm). In specimens with portal area counts, 67% had four or more than four portal areas.

Development of hepatocellular carcinoma

The median follow-up time was 29 (range, 3-133) months. Of 147 patients, 48 (33%) developed HCC during the follow-up period. For those who developed HCC, in 43 cases the segment where this occurred correlated with the nodular segment, whereas in five cases its occurrence differed from the segments with the index liver nodules. Regarding the diagnosis of HCC, 15 cases were diagnosed by histology (confirmed by resection or needle-guided biopsy), and 26 and 7 cases were diagnosed by characteristic imaging features and diagnostic AFP levels, respectively. The overall cumulative occurrence rates of HCC were 18% at 1 year, 22% at 2 years, 27% at 3 years, and 30% at 4 years. The cumulative HCC incidence rates according to baseline histology are listed in Table 2.

To elucidate predictive factors leading to the development of HCC from liver nodules, both patient and tumour characteristics were analysed by univariate Cox regression analysis. Two clinical

parameters were predictive, namely: age and HBV carrier status (HBsAg positive). Regarding laboratory parameters, nodules with dysplastic changes were associated with higher probabilities of developing HCC—LGDNs had a 4- to 5-fold (95% confidence interval [CI], 2.13-10.22; P<0.001) increased risk compared with RNs; the risk increased 8-fold (95% CI, 3.86-17.81; P<0.001) if high-grade dysplastic changes were evident. Figure 1 shows the Kaplan-Meier analysis of time to HCC development in patients with RN, LGDN, and HGDN. For the biochemical parameters, increased levels of AFP and bilirubin, as well as prothrombin times were positively associated with higher rates of HCC development, whereas serum albumin and platelet counts were negatively associated with risk of HCC development. The predictive properties of sex, history of HCC, and imaging features (including USG and CT) were not statistically significant. Table 3 shows the results of univariate analysis of demographic, clinical, biochemical, and histological variables in relation to the development of HCC.

Multivariate analysis by the Cox regression model was performed to adjust for confounding effects on each variable, and revealed that only three baseline characteristics were independent predictors of HCC development. They were age, baseline AFP level, and dysplastic histological changes. Compared with RN, LGDN had 2.77-fold (95% CI, 1.18-6.46)

TABLE 1. Baseline characteristics of regenerative nodules (RN), low-grade dysplastic nodules (LGDN), and high-grade dysplastic nodules (HGDN)*

Characteristic†	RN (n=74)	LGDN (n=34)	HGDN (n=39)	P value
Male	50 (68%)	27 (79%)	33 (85%)	0.109‡
Age (years)	54 (22-81)	57 (42-83)	63 (44-79)	<0.001§
HBsAg positive (+ve:-ve)	40:34	29:5	28:11	0.004‡
HBeAg positive (+ve:-ve)	6:63	7:23	3:32	0.092‡
HCV antibody positive (+ve:-ve)	10:50	4:15	6:25	0.893‡
Alcoholic (drinkers:non-drinkers)	8:58	1:29	1:35	0.069‡
AFP (ng/mL)	4.0 (1.4-47.6)	7.1 (1.9-159.0)	12.6 (1.3-160.0)	0.001§
Bilirubin (mmol/L)	12 (4-65)	15 (8-102)	18 (4-139)	0.011§
Albumin (g/L)	40 (17-51)	38 (25-44)	38 (15-46)	0.309§
Prothrombin time (sec)	12 (10-15.3)	12 (11-18)	13 (11-18)	0.012§
Platelet count (x 10^9 /L)	194 (72-606)	126 (60-284)	112 (38-382)	<0.001§
Child-Pugh stage				
A	62 (84%)	25 (74%)	33 (85%)	0.089‡
B	11 (15%)	5 (15%)	4 (10%)	
C	1 (1%)	4 (12%)	2 (5%)	
Size (cm)	1.8 (0.5-6.9)	2.0 (1.0-5.7)	3.0 (1.3-6.8)	<0.001§
USG (hyper-echoic:hypo-echoic)	24:31	7:19	12:19	0.352‡
CT (hypo/iso:hyper-attenuating)				
Plain	45:4	18:5	28:4	0.267‡
Arterial	29:20	10:13	18:14	0.450‡
Portal venous	38:11	14:9	24:8	0.317‡
Interventions (yes:no)	6:68	8:26	29:10	<0.001‡

* Data are shown as No. or median (range)
† HBsAg denotes hepatitis B surface antigen, HBeAg hepatitis B e antigen, HCV hepatitis C virus, AFP alpha-fetoprotein, USG ultrasonography, and CT computed tomography
‡ Chi squared or Fisher's exact test
§ Kruskal-Wallis test

TABLE 2. Cumulative numbers with hepatocellular carcinoma*

Nodule	First year	Second year	Third year	Fourth year
RN (n=74)	2 (3%)	4 (5%)	7 (9%)	9 (12%)
LGDN (n=34)	10 (29%)	12 (35%)	13 (38%)	15 (44%)
HGDN (n=39)	15 (38%)	16 (41%)	20 (51%)	20 (51%)

* RN denotes regenerative nodules, LGDN low-grade dysplastic nodules, and HGDN high-grade dysplastic nodules

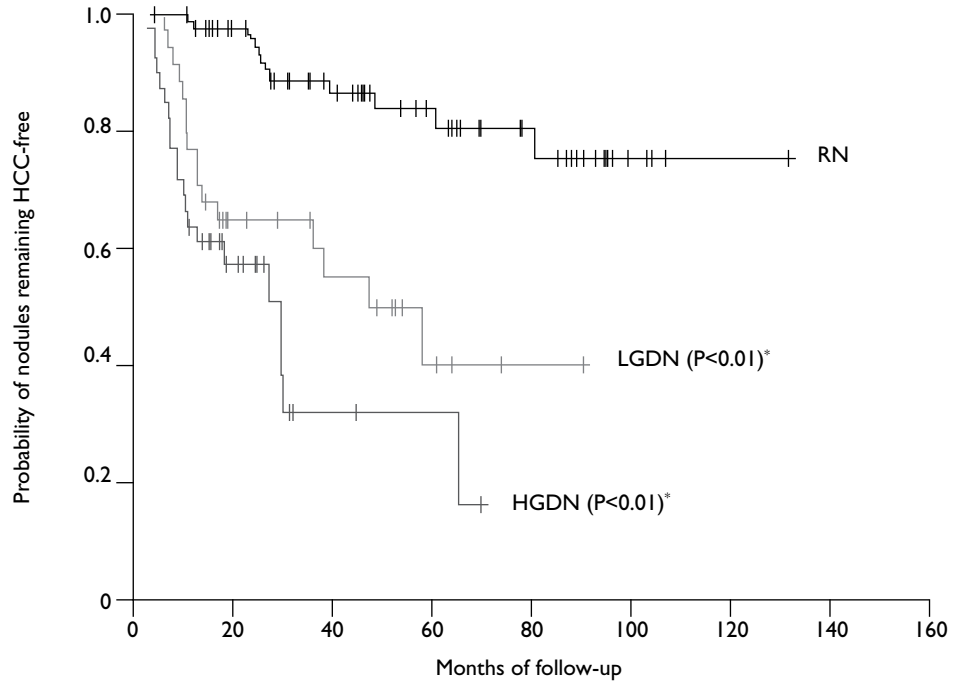
increased risk of development of HCC. The risk for the HGDN group was even higher with a relative risk of 5.18-fold (95% CI, 2.29-11.74) [Table 4]. After adjusting for other covariates, positive correlations with HBsAg status, interventions, serum albumin and bilirubin levels, prothrombin times, and platelet counts became insignificant predictors.

Survival

Thirty-seven (25%) of the 147 patients died during the follow-up period, 23 (62%) due to HCC, nine (24%) due to liver cirrhosis, and five (14%) from non–liver-related causes. The overall cumulative mortality rates were 5% at 1 year, 13% at 2 years, 16% at 3 years, and 20% at 4 years. The 3-year cumulative mortality rate in the HGDN group (31%) was higher than that in the LGDN and RN groups, with respective mortality rates of 21% and 7%. Figure 2 demonstrates the Kaplan-Meier analysis of survival for patients in the RN, LGDN, and HGDN groups. The multivariate Cox proportional hazard model showed that age (odds ratio [OR]=1.12; 95% CI, 1.07-1.17), male gender (OR=9.82; 95% CI, 2.01-47.92), prothrombin time in seconds (OR=1.63; 95% CI, 1.26-2.10), nodules histology, and hyper-echoic appearance on USG (OR=0.21; 95% CI, 0.07-0.50) were independent predictors of survival. The LGDN group had a 5.7-fold higher risk of death compared with the RN group (95% CI, 1.8-18.2). The mortality risk increased to 7.0-fold (95% CI, 2.2-22.8) if high-grade dysplastic changes were evident.

Discussion

In the present study, the role of DNs as a risk factor for HCC was investigated. Although patients with DNs were generally older and had poorer baseline liver function, the presence of such histology stood out as an independent risk factor for HCC development



	HCC development time (months)						
	0	20	40	60	80	100	120
No. at risk							
RN	74	62	36	26	15	3	1
LGDN	34	16	11	3	1		
HGDN	39	13	3	2			
No. with HCC							
RN	0	2	8	9	10	11	11
LGDN	0	12	14	16	16		
HGDN	0	16	20	20			

* Log rank test

FIG 1. Cumulative probability of liver nodules remaining hepatocellular carcinoma (HCC)–free according to histological type
RN denotes regenerative nodules, LGDN low-grade dysplastic nodules, and HGDN high-grade dysplastic nodules

even after adjusting for these differences; having an HGDN had a 5.2-fold higher risk than having a RN. The risk was even higher if associated with advanced age and increased AFP level. In addition, the presence of LGDNs and HGDNs indicated a higher probability of death after adjustment for other covariates. Age, male sex, prolonged prothrombin time, and hypoechoic appearance on USG also predicted mortality in patients with liver nodules.

In the current series, 48 (33%) of 147 of the hepatic lesions transformed into HCC. Our findings were similar to those of Borzio et al,[8] who reported an HCC rate of 31% at 3 years after follow-up of 90 patients with liver nodules. Our results were expected because the patient demographic data of the current study were similar to Borzio et al's series,[8] with most patients belonging to Child-Pugh A liver status. The causes of liver disease, however, were different in

TABLE 3. Univariate analysis of possible predictors of hepatocellular carcinoma (HCC) development by the Cox regression method*

Predictor	No. of patients/total	RR	95% CI	P value
Male	110/147	2.18	0.98-4.85	0.057†
Age	NA	1.04	1.01-1.06	0.005‡
HBsAg positive	97/147	2.13	1.08-4.20	0.028†
HBeAg positive	16/134	1.34	0.56-3.20	0.510†
HCV antibody positive	20/110	1.53	0.71-3.30	0.281†
Alcoholic	9/132	0.24	0.03-1.74	0.157†
AFP	NA	1.02	0.03-1.04	<0.001‡
Bilirubin	NA	1.02	1.01-1.03	0.005‡
Albumin	NA	0.94	0.90-0.98	0.004‡
Prothrombin time	NA	1.43	1.19-1.71	<0.001‡
Child-Pugh stage				
B vs A	20	1.76	0.84-3.67	0.131†
C vs A	7	2.61	0.79-8.61	0.115†
Platelet count	NA	0.99	0.98-1.00	<0.001‡
Hyper-echoic in USG	43/112	0.99	0.51-1.92	0.966†
Hyper-attenuating in plain CT	13/104	1.23	0.47-3.17	0.673†
Hyper-attenuating in arterial phase CT	47/104	0.79	0.41-1.51	0.467†
Hyper-attenuating in portal venous phase CT	28/104	0.84	0.38-1.84	0.663†
Size	NA	1.22	1.00-1.50	0.051‡
Histology				
LGDN	34	4.67	2.13-10.22	<0.001†
HGDN	39	8.29	3.86-17.81	<0.001†
Intervention	43/147	1.99	1.11-3.60	0.021†

* RR denotes relative risk, CI confidence interval, NA not available, HBsAg hepatitis B surface antigen, HBeAg hepatitis B e antigen, HCV hepatitis C virus, AFP alpha-fetoprotein, USG ultrasonography, CT computed tomography, LGDN low-grade dysplastic nodule, and HGDN high-grade dysplastic nodule

† Data were analysed as categorical variables

‡ Data were analysed as continuous variables

TABLE 4. Positive findings of multivariate analysis of factors associated with hepatocellular carcinoma development (Cox proportional hazard model)*

Predictor	Hazard ratio	95% CI	P value
Age	1.04	1.01-1.07	0.007†
AFP	1.02	1.02-1.03	<0.001†
Histology			
LGDN	2.77	1.18-6.46	0.019‡
HGDN	5.18	2.29-11.74	<0.001‡

* CI denotes confidence interval, AFP alpha-fetoprotein, LGDN low-grade dysplastic nodule, and HGDN high-grade dysplastic nodule

† Data were analysed as continuous variables

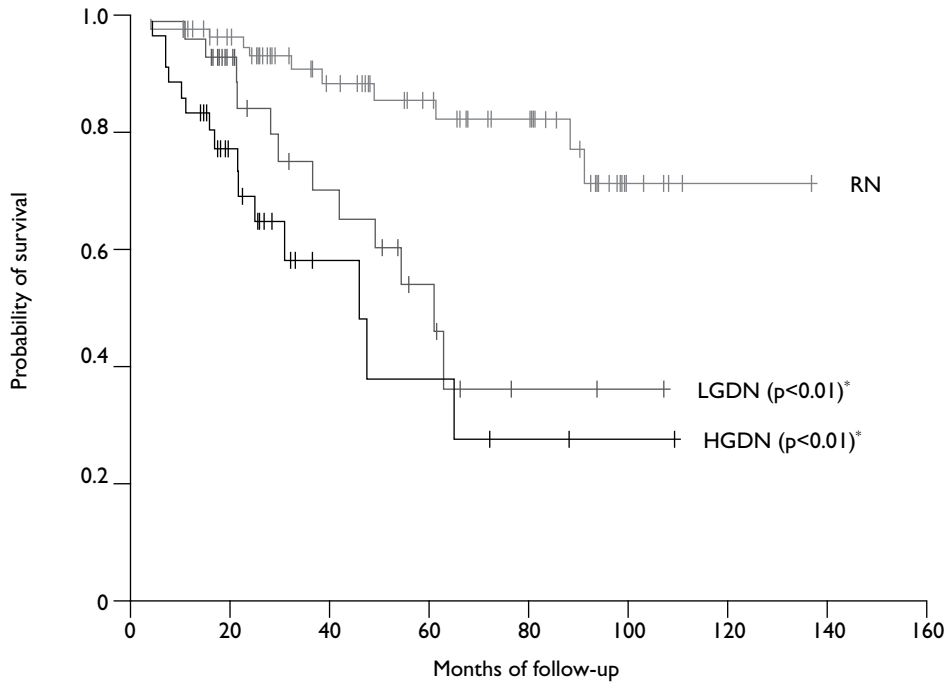
‡ Data were analysed as categorical variables

the two studies. In Borzio et al's series,[8] the most common cause of liver cirrhosis was HCV infection (51.1%), as opposed to HBV infection–associated cirrhosis in the current study. Besides, our data showed that after adjusting for other covariates, liver disease aetiology was not an independent predictor of HCC development. These results may suggest that once liver macronodules are formed, they become strong predictors of an increased risk of malignancy, regardless of the underlying cause of cirrhosis.

In our study, age was also an independent risk factor for HCC. Previous studies proved that age was a risk factor of HCC in patients with HBV[9] and cirrhosis.[10] Age probably reflects the duration of underlying liver disease regardless of aetiology. Arguably, the longer the duration of insults, the higher the risk of cumulative genetic damage exceeding the threshold leading to cell growth aberration.

Elevated AFP level is a recognised marker of HCC development. Tanaka et al[11] reported a follow-up study involving 100 patients with cirrhosis, and suggested that levels higher than 20 ng/mL increased the risk of HCC development. Another Japanese study involving a larger sample size (n=917) showed a similar result, the HCC development risk in patients with AFP levels of more than 20 ng/mL was 3.2-fold (95% CI, 1.7-6.0) higher than in those with lower levels.[10] Our data were in keeping with these Japanese results, with elevated AFP levels being an independent predictor of HCC development after adjustment for other confounding variables in the multivariate analysis.

Correlation of pre-biopsy imaging features with histological findings indicated that both USG and CT imaging yielded suboptimal accuracy for these liver nodules. This was consistent with previous studies showing that USG and CT features are not very helpful in differentiating between these liver nodules, as hepatic dysplasia showed diverse imaging characteristics.[12] Another of our findings meriting



	Follow-up time (months)						
	0	20	40	60	80	100	120
No. at risk							
RN	74	63	37	26	14	4	1
LGDN	34	20	14	4	2	1	
HGDN	39	19	6	4	2	1	
No. of deaths							
RN	0	2	8	8	8	10	10
LGDN	0	4	8	12	12	12	
HGDN	0	9	12	14	15	15	

* Log rank test

FIG 2. Cumulative probability of survival according to histological type
HCC denotes hepatocellular carcinoma, RN regenerative nodules, LGDN low-grade dysplastic nodules, and HGDN high-grade dysplastic nodules

discussion was the 'protective' role of hyper-echoic USG imaging features on patient survival. A previous study had found a significant correlation between the echogenicity of DNs and the differences in fat content between nodules and liver parenchyma.[13] The echogenicity of a nodule determined on sonography appears to reflect mainly fatty, clear-cell, and small-cell changes with increased nuclear crowding. Terasaki et al[14] showed that these histological features are predictive of malignant transformation of non-malignant liver nodules. Therefore, it appeared that hyper-echoic DNs that showed abundant fatty change were likely to progress to HCCs. In contrast to the previous study, we did not show any predictive role of a hyper-echoic appearance on HCC development. Moreover, patients having a hyper-echoic liver nodule had a better chance of survival, which was unexpected. Conceivably, patients with RNs and DNs belong to two distinct groups with their own USG echogenic patterns. Since we had included all three groups (RNs, LGDNs, and HGDNs) into analysis, the positive predictive role of USG echogenicity on survival may relate to the presence of RNs. Subgroup analysis of patients with DNs (including LGDNs and HGDNs) confirmed that hyper-echoic USG had no association with patient survival.

Interestingly, not all DNs developed into HCCs. In the current study, 19 patients with LGDNs and two with HGDNs decreased in size or remained static without any intervention. In addition, similar to other reported case series,[15] not all DNs develop into HCC. Such findings do not contradict the 'multistep process' hypothesis for hepatocarcinogenesis[4] with DN as a precursor of HCC development. It is probable that an additional 'hit' is required for a precancerous lesion to become a genuine cancer.

Data for prediction of HCC development and survival of patients with liver nodules found during HCC screening are important for several reasons. First, with a better understanding of the clinical course of different types of liver nodules, both patients and clinicians can make more informed treatment choices. Regenerative nodules, in general, have a more benign course and therefore monitoring and avoidance of invasive treatment seems appropriate. Dysplastic nodules, on the other hand, command more aggressive approaches. Second, more accurate prediction of survival can help clinicians decide when to refer a patient for liver transplantation. It has been suggested that patients with HGDNs should be enrolled on liver transplant waiting lists, since they are also associated with subsequent HCC development and decreased survival.[16] In our study, the 5-year survival of patients with HGDNs was less than 50%, which was much less than the commonly quoted 70 to 80% figure for liver transplant recipients.[17]

Our study had intrinsic weaknesses. First, the criteria of selecting lesions for biopsy were not standardised. In our centre, we generally follow the US recommendations for managing patients with liver nodules. Accordingly, biopsy of the lesions was considered if the diagnosis remained uncertain after two dynamic imaging sessions, in addition to an equivocal serum AFP level.[7] Since our study was retrospective, many of our cases were recruited before these recommendations were widely practised, and the criteria were not strictly followed. Second, the retrospective nature of our study limited complete data collection. A history of habitual alcoholic intake was only recorded in some of the cases. Moreover, measurement bias could not be prevented as the follow-up regimen of patients with liver nodules was not standardised, and baseline histology was known at the beginning. Third, sampling errors during liver biopsy could not be eliminated; such errors lead to problem due to misclassification of patients. In the study by Kobayashi et al,[18] the investigators regularly recorded the USG-guided biopsy procedure by video. Since we had no video recording of USG imaging during biopsies, sampling errors could not be retrospectively estimated. Finally, two important predictors of HCC development in HBV carriers were not collected and analysed in this study, namely HBV DNA level and a family history of HCC. From data of the REVEAL study,[19] HBV carriers with greater than 10^5 copies/mL of HBV DNA at enrolment had a 10-fold risk of developing HCC compared with carriers with fewer than 10^4 copies/mL. Another study from Haimen city in China showed a similar result.[20] In our study, we did not routinely check the HBV DNA levels during the biopsy period and therefore these important parameters were not measured and adjusted for during analysis. Similarly, a family history of HCC was also not recorded in the case notes in most instances and so was not analysed, although its predictive role in HCC has been reported among HBV carriers.[21]

Conclusions

Better prediction of HCC development and survival in patients with non-malignant liver nodules is important. It provides information for clinicians in formulating management plans for patients with such lesions with respective screening. We conclude that dysplastic histology, elevated AFP levels, and age are independent predictors of HCC development. Dysplastic change is also a significant predictor of survival. More prospective follow-up studies, which include baseline HBV DNA measurements and family history taking of HCC, are urgently needed to refine the prediction model.

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