Original Paper

Direct-Acting Antiviral Agents Reduce the Risk of Malignant Transformation of Hepatobiliary Phase-Hypointense Nodule without Arterial Phase Hyperenhancement to Hepatocellular Carcinoma on Gd-EOB-DPTA-Enhanced Imaging in the Hepatitis C Virus-Infected Liver

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Keywords

Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid · Hepatobiliary phase-hypointense nodule without arterial phase hyperenhancement · Hepatocellular carcinoma · Direct-acting antivirals · Hepatitis C virus

Abstract

Background and Aims: Hepatobiliary phase-hypointense nodules without arterial phase hyperenhancement (HHNs without APHE) on gadolinium-ethoxybenzyl-diethylenetriamine-enhanced magnetic resonance imaging (Gd-EOB-DTPA-enhanced MRI) are considered to be dysplastic nodules or early hepatocellular carcinoma (HCC) and have high risk of undergoing malignant transformation and progression to hypervascular HCC. The aim of this study was to evaluate the clinical outcome of HHNs without APHE diagnosed by Gd-EOB-DTPA-enhanced MRI before the eradication of HCV by direct-acting antiviral agents (DAAs). **Methods:** We retrospectively investigated 221 consecutive patients with HCV infection who were treated with DAAs. Thirty patients with 65 HHNs without APHE were enrolled in a sustained virologic response (SVR) cohort and 22 with 43 HHNs without APHE who did not receive DAAs or had failed HCV eradication therapy were enrolled in a non-SVR cohort. Fifty-seven percent of patients in the SVR group and 64% of those in the non-SVR group had a history of HCC. The primary endpoint of this study was the development of hypervascular HCC from HHNs with-

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out APHE detected on imaging. The cumulative incidence and relative risk of progression to hypervascular HCC in relation to clinical characteristics were compared between the two cohorts. **Results:** The 2-year cumulative incidence of progression to hypervascular HCC was 8.5 and 21.9% in the SVR and non-SVR cohorts, respectively. There was a significant reduction in progression of HHNs without APHE to HCC after the eradication of HCV ($p = 0.022$, log-rank test). Multivariate Cox regression analysis identified hyperintensity on T2-weighted images (relative risk 14.699, $p < 0.001$) and achieving SVR (relative risk 0.290, $p = 0.043$) as independent factors associated with the risk of HCC. During follow-up, 6 (9.2%) of the HHNs without APHE in the SVR cohort became undetectable on hepatocyte-phase images. **Conclusions:** Eradication of HCV by DAAs could reduce the hypervascularization rate of HHNs without APHE, and some of these nodules disappeared.

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Introduction

Hepatocellular carcinoma (HCC) is one of the deadliest cancers worldwide [1]. Carcinogenesis in the liver is generally considered to occur as a result of accumulation of multiple genetic/epigenetic changes, and liver nodules can be classified as non-malignant cirrhotic, premalignant dysplastic, or malignant HCC [2].

Characteristic differences in arterial/portal flow have been identified between non-malignant, premalignant, and malignant liver nodules on dynamic computed tomography (CT) and magnetic resonance imaging (MRI) studies [3–5]. CT during hepatic angiography or arteriportography is recognized as the most sensitive modality for assessing the vascularity of HCC/premalignant lesions in the cirrhotic liver [5]. These reports indicate that increased vascularity in the arterial phase accompanied by loss of portal venous flow in the portal phase should be the gold standard for the diagnosis of HCC. Moreover, gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA), a magnetic resonance (MR) contrast agent used to evaluate hepatocyte function according to organic anion transporter polypeptide 1B3 (OATP1B3) activity, has taken on an increasingly important role in diagnostic imaging of the liver. The reason for the increased diagnostic role of Gd-EOB-DTPA is that the expression of OATP1B3 decreases in premalignant dysplastic nodules, which can be detected as hypointense signals on hepatobiliary phase (HBP) images [4, 6, 7]. Precise analysis of radiological images and pathological findings in biopsy specimens has shown that a hypovascular, HBP-hypointense nodule on Gd-EOB-DTPA-enhanced MR images corresponds histologically to early well-differentiated HCC (without hypervascularity) or premalignant dysplastic nodules [8, 9]. The terminology proposed by the Liver Imaging Reporting and Data System for this type of nodule is “hepatobiliary phase-hypointense nodule without arterial phase hyperenhancement (HHN without APHE)” [10].

HHNs without APHE have a high risk of undergoing malignant transformation and progression to HCC, and so are monitored carefully following diagnosis in clinical practice. Previous reports indicate that HHNs without APHE measuring greater than 10–15 mm [11–13], those that show hyperintensity on T2-weighted images (T2WI) [14, 15] and diffusion-weighted images [16, 17], and those that show fatty changes [11, 18] are at high risk of progression to HCC.

Hepatitis C virus (HCV) infection is one of the major causes of liver cirrhosis and HCC worldwide. Recent advances in our understanding of the molecular aspects of HCV replication have led to the successful development of direct-acting antiviral agents (DAAs) that target the HCV proteins NS3, NS5A, and NS5B [19]. Approximately 98% of patients with HCV infection now achieve a sustained virologic response (SVR) [19]. A meta-analysis of the results

Fig. 1. Flow chart showing the process of patient selection in the SVR cohort (left panel) and the non-SVR cohort (right panel).

of interferon (IFN)-based regimens revealed an association between eradication of HCV and a reduced risk of liver-related complications, including development of HCC [20]. Achievement of SVR with IFN-free DAA therapy has also been reported to reduce the risk of HCC [21]. However, outcomes in several studies have been conflicting [22–24] and little is known about the effect of eradication of HCV on progression of HHNs without APHE to HCC. The aim of this study was to evaluate the clinical outcome of HHNs without APHE diagnosed by using Gd-EOB-DTPA-enhanced MRI before the eradication of HCV by DAA therapy.

Materials and Methods

Patients

We retrospectively identified 221 consecutive patients with HCV infection who were treated with DAAs at Kanazawa University Hospital from November 2014 to April 2016. The inclusion and exclusion criteria are shown in Figure 1. Patients who were serum hepatitis B surface antigen positive, had anti-human immunodeficiency virus antibodies, or other causes of hepatocellular injury (such as alcoholic liver injury, autoimmune hepatitis, primary biliary cholangitis, or a history of treatment with hepatotoxic drugs) were excluded. Patients with early-stage HCC who had undergone radical hepatic resection or radiofrequency ablation within the preceding 3 months were enrolled. Given the retrospective nature of the study, we examined EOB-MRI findings in patients at high risk of developing HCC, that is, those with a past history of HCC, those with advanced liver fibrosis, and those with HHN without APHE.

Thirty patients with 65 HHNs without APHE on Gd-EOB-DTPA-enhanced MRI were enrolled in the SVR cohort (14 men, 16 women; mean age 70.6 years; cirrhosis 77%; past history of HCC 57%; Child-Pugh class A liver cirrhosis 100%). The median observation period was 3 months (range 0–9; starting from the time of acquisition of the initial MR images until the start of DAA therapy). The treatment regimens in the SVR cohort were as follows: asunaprevir and daclatasvir for 24 weeks ($n = 14$); sofosbuvir and ledipasvir for 12 weeks ($n = 13$); sofosbuvir and ribavirin for 12 weeks ($n = 2$), and ombitasvir and paritaprevir in combination with ritonavir for 12 weeks ($n = 1$). SVR was defined as no HCV RNA detectable in serum 24 weeks after the cessation of treatment. Twenty-two patients with 43 HHNs without APHE who did not receive antiviral therapy or in whom IFN-based therapy failed to eradicate HCV from April 2008 to June 2009 were enrolled

Table 1. Baseline characteristics and HCC details**a** Baseline characteristics of patients and nodules enrolled in the study

Parameter	SVR	Non-SVR	<i>p</i> value
<i>Patients</i>	(<i>n</i> = 30)	(<i>n</i> = 22)	
Sex (male/female)	14 (47)/16 (53)	13 (59)/9 (41)	0.38
Age, years	70.6±9.1	72.7±6.0	0.25
Cirrhosis (no/yes)	7 (23)/23 (77)	5 (23)/17 (77)	0.96
HCV serogroup (1/2/unknown)	28/2/0	16/3/3	0.59
Platelets, ×10 ⁴ /μL	10.3±4.5	10.6±5.0	0.82
Aspartate transaminase, IU/L	51.0±19.2	59.3±31.5	0.28
Alanine transaminase, IU/L	42.2±18.6	51.7±36.1	0.27
Albumin, g/dL	3.54±0.44	3.74±0.49	0.14
Total bilirubin, mg/dL	0.90±0.44	0.90±0.33	0.51
Prothrombin activity, %	75.8±11.2	78.7±11.9	0.38
Alpha-fetoprotein, ng/mL	52.6±128.8	183.4±443.6	0.19
Past history of HCC (yes)	17 (57)	14 (64)	0.61
<i>Nodules</i>	(<i>n</i> = 65)	(<i>n</i> = 43)	
Nodule size, mm	7.8±2.4	8.4±2.3	0.20
Monitoring period, months	22.3±8.3	25.2±15.5	0.33
Hyperintensity on T2WI (yes)	6 (9.2)	2 (4.7)	0.61

Data are presented as *n* (%) or the mean ± SD. *p* values were determined by χ^2 test or unpaired *t* test. HCC, hepatocellular carcinoma; SVR, sustained virologic response; T2WI, T2-weighted imaging.

b Details of past HCC

	SVR	Non-SVR
Size (≤2 cm/>2 cm)	4/13	12/2
Number (1/≥2)	9/8	5/9
Vascular invasion	0	0
C-stage (I/II/III)	7/8/2	3/10/1
Treatments, <i>n</i> (median)	2 (1–7)	2 (1–6)
Hepatectomy	2	1
RFA	14	13
TACE	2	4

The clinical stage is based on the general rules for the clinical and pathological study of primary liver cancer published by the Liver Cancer Study Group of Japan. Size, number, and C-stage were determined at the time of the last treatment for HCC. Treatments refers to the total number received. Treatment was curative in all cases. RFA, radiofrequency ablation; TACE, transarterial chemoembolization.

as a non-SVR cohort (13 men, 9 women; mean age 72.7 years; cirrhosis 77%; past history of HCC 64%; Child-Pugh class A liver cirrhosis 100%). The baseline characteristics of the patients and nodules in this cohort are shown in Table 1. The presence of cirrhosis was determined according to histological findings, except in 1 case.

The study protocol was approved by the institutional ethics committee at Kanazawa University Hospital. The requirement for informed consent was waived in view of the retrospective observational nature of the study.

Imaging Analysis

MRI of the liver was performed in all patients using a 1.5- or 3-T MRI system (Signa HDx; GE Healthcare, Milwaukee, WI, USA). We obtained fat-saturated three-dimensional gradient echo T1-weighted images and fat-saturated fast spin-echo T2WIs [25]. In all cases, an intravenous bolus injection of Gd-EOB-DTPA

(Primovist®; Bayer Schering Pharma, Berlin, Germany) was administered as the contrast agent at a dose of 25 µmol/kg body weight at a flow rate of 1 mL/s, followed by a 20-mL saline flush. Dynamic imaging was performed using a fat-suppressed three-dimensional T1-weighted GRE sequence with parallel imaging (liver acquisition with volume acceleration, LAVA; GE Medical Systems, Milwaukee, WI, USA) during breath holding. The following imaging parameters were used: TR/TE, 3.3–3.6 ms/1.6 ms; flip angle, 8–15° or 12–15°; acquisition time, 12–18 s; field of view, 42 × 42 cm; matrix, 320 × 192 to 384 × 256, interpolated to 512 × 512; thickness, 4.2 mm; overlap, 2.1 mm, and ASSET acceleration, 2.0. Arterial phase images were obtained 20–35 s after the injection of contrast material and HBP images at 20 min. The same MRI scanner and image acquisition conditions were used in the SVR and non-SVR cohorts.

All images were interpreted by experienced board-certified radiologists and double-checked independently by experienced board-certified hepatologists. The HHNs without APHE were delineated from the surrounding liver tissue as round lesions that were hypointense on both axial and coronal Gd-EOB-DTPA-enhanced HBP MRI, were ≥5 mm in maximum diameter in the axial plane as measured on HBP images, and were hypovascular during the arterial phase. Dynamic CT images for the portal venous/delayed phase were also analyzed in small (6-mm) nodules to reduce the risk of any HHNs without APHE being missed in view of the small sample size.

The primary endpoint was progression of HHNs without APHE to hypervascular HCC as confirmed by a combination of APHE and washout on portal venous/transitional phases by Gd-EOB-DTPA-enhanced MRI or portal venous/delayed phase by dynamic CT. Nodular enlargement was defined as an increase in diameter of ≥3 mm during the monitoring period. A nodule was deemed to have disappeared if its signal intensity (SI) during the HBP phase of Gd-EOB-DTPA-enhanced MRI was found to be similar to that of the background liver or was unclear on at least two studies performed during the monitoring period.

We assessed the baseline radiological characteristics of each nodule in the SVR cohort by the enhancement ratio (ER) and Delta T1. The SI of the tumor was measured within the operator-defined region of interest (ROI) in the precontrast phase and HBP. The ROI of the tumor was defined as the maximum oval or round area at the level of the largest diameter of the tumor. The SI of the adjacent background liver was also measured in an ROI of the same size as the nodule ROI, avoiding the major portal tracts and artifacts. For absolute quantitative evaluation of the ER, we calculated the static T1 value. The static T1 value before enhancement (T1pre) was calculated as follows: $\exp(-TR/T1pre) = (SI_A \times \sin\beta - SI_B \times \sin\alpha) / (SI_A \times \sin\beta \times \cos\alpha - SI_B \times \sin\alpha \times \cos\beta)$. SI_A and SI_B represent the SI in flip angles α and β , respectively. The enhanced images were obtained with the same parameters using flip angle α . The T1 value after enhancement (T1post) was then calculated as: $\exp(-TR/T1post) = \{SIpre \times [1 - \exp(-TR/T1pre) \times \cos\alpha] + SIpost \times [\exp(-TR/T1pre) - 1]\} / \{SIpre \times [1 - \exp(-TR/T1pre) \times \cos\alpha] + SIpost \times [\exp(-TR/T1pre) - 1] \times \cos\alpha\}$. We calculated the ER as $(1/T1post - 1/T1pre) / 1/T1pre$ [26, 27]. Our findings indicated that the ER on the Gd-EOB-DTPA-enhanced MR image was decreased as a result of the poorly differentiated morphology of HCC and the decreased expression of OATP1B3 [26]. We analyzed correlations between the ER and radiological changes in the nodule after achievement of an SVR. Delta T1 was calculated as follows: $|\text{post-T1 value} - \text{pre-T1 value}|$.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows software (version 23.0; IBM Corp., Armonk, NY, USA). Continuous variables are expressed as the mean and standard deviation, and the categorical variables as the count and percentage. Continuous variables, such as tumor size and duration of observation were compared using the Mann-Whitney U test and categorical variables were compared using Fisher's exact test or the χ^2 test. The incidence of progression of HHNs without APHE to hypervascular HCC was evaluated using Kaplan-Meier analysis, and the log-rank test was used for the statistical analysis. The relative risk of progression to HCC associated with various clinical factors was analyzed using Cox's proportional hazards regression analysis. Tukey's multiple comparison test was used to assess the correlation between changes in the nodules seen on imaging and the ER. A p value <0.05 was considered statistically significant.

Results

Baseline Characteristics of Patients and Nodules

The baseline characteristics of the patients in the SVR cohort and those in the non-SVR cohort are summarized in Table 1. A degree of selection bias may have been introduced by

Fig. 2. Representative images of an HBP-hypointense nodule without APHE on Gd-EOB-DTPA-enhanced MRI at the time of the initial diagnosis (upper panels) and 9 months later (lower panels). Hypervascular HCC developed from this nodule even after achievement of an SVR.

Table 2. Clinical outcome of HHNs without APHE on Gd-EOB-DTPA-enhanced MRI during the monitoring period

	SVR (n = 65)	non-SVR (n = 43)
Hypervascularization	5 (7.7)	15 (34.9)
Enlargement without hypervascularization	4 (6.2)	6 (13.9)
Unchanged in size	50 (76.9)	22 (51.2)
Disappeared	6 (9.2)	0 (0.0)
Monitoring period, months	21.2 (4.2–39.9)	22.6 (4.6–52.3)

Data are presented as n (%) or the median (range). SVR, sustained virologic response.

the likelihood of non-treatment in patients who were elderly, those who had advanced fibrosis or a history of HCC, and those who could not tolerate IFN because of adverse events. Therefore, we compared the baseline characteristics between the two cohorts. There was no between-group difference in sex, age, clinical data, proportion of patients with liver cirrhosis, or past history of HCC. There was also no statistically significant difference in the characteristics of the HHNs without APHE between the groups.

Cumulative Incidence of Progression to HCC

The median duration of monitoring after the initial Gd-EOB-DTPA-enhanced MRI examination was 21.2 months (range 4.2–39.9) in the SVR cohort and 22.6 months (range 4.6–52.3) in the non-SVR cohort ($p = 0.326$). Dynamic CT or MRI was repeated every 3–6 months in all patients. The median interval between EOB-MRI examinations was 8.0 months (range 3.0–24.0). Five (7.7%) of the 65 HHNs without APHE in the SVR cohort progressed to HCC during follow-up; in contrast, 15 (34.9%) of 43 HHNs without APHE progressed to HCC in the non-SVR cohort (Table 2). Representative MR images of an HHN without APHE that progressed

Fig. 3. Cumulative rates for progression from an HBP-hypointense nodule without APHE to hypervascular HCC. The overall cumulative incidence of progression was 4.7% at 1 year and 8.5% at 2 years in the SVR cohort, and 14.7 and 21.9%, respectively, in the non-SVR cohort ($p = 0.022$, log-rank test).

to hypervascular HCC are shown in Figure 2. The overall cumulative incidence of progression of HHNs without APHE to HCC was 4.7% at 1 year and 8.5% at 2 years in the SVR cohort, and 14.7 and 21.9% in the non-SVR cohort, respectively. Eradication of HCV significantly suppressed the progression of HHNs without APHE to HCC ($p = 0.022$, log-rank test; Fig. 3). Progression to HCC was not observed 20 months or later after the initial diagnosis of HHNs without APHE on MRI in the SVR cohort. In the SVR cohort, there was no significant difference in serum alpha-fetoprotein level between patients who did and did not develop HCC from HHNs without APHE at 24 weeks after the administration of DAAs ($p = 0.23$).

In univariate analysis, 5 of 13 clinical factors were significantly associated with increased risk of progression of HHNs without APHE to HCC: male sex, large nodule size, hyperintensity on T2WI, lack of SVR with DAA therapy, and high serum alanine transaminase level at initial screening (Table 3). Multivariate Cox regression analysis identified two independent factors associated with risk of HCC, namely hyperintensity on T2WI (relative risk 14.699; $p < 0.001$, 95% confidence interval 3.827–56.450) and achievement of SVR with DAA therapy (relative risk 0.290; $p = 0.043$, 95% confidence interval 0.087–0.961). Kaplan-Meier survival analysis indicated that achievement of SVR with DAA therapy strongly suppressed the progression to HCC of HHNs without APHE that were isointense or hypointense on T2WI (Fig. 4).

Development of HCC according to History of HCC

We analyzed the outcomes of HHNs without APHE according to whether or not there was a past history of HCC. Detailed information on the characteristics of HCC and their treatment are shown in Table 1b. In patients with a history of HCC, achievement of SVR with DAA therapy was associated with a statistically significant decrease in the risk of development of HCC ($p = 0.037$, log-rank test; Fig. 5). In contrast, there was no statistically significant difference in the rate of progression to HCC in patients without a history of HCC according to whether or not there was an SVR ($p = 0.13$, log-rank test).

Disappearance of Some HHNs without APHE after Achievement of SVR with DAA Therapy

During follow-up, 6 HHNs without APHE (9.2%) in the SVR cohort disappeared and could no longer be seen on Gd-EOB-DTPA-enhanced HBP MR images (Fig. 6). No such nodules were

Fig. 4. Cumulative rates for progression of an HBP-hypointense nodule without APHE to hypervascularization according to the findings on T2WI.

Table 3. Univariate and multivariate analysis of clinical characteristics and risk of progression of HHNs without APHE on Gd-EOB-DPTA-enhanced MRI to hypervascular HCC

Parameter	HR	95% CI	p value
<i>Univariate analysis</i>			
Sex (male/female)	3.04	1.18–7.83	0.02
Age (years; continuous variable)	1.00	0.95–1.06	0.98
Cirrhosis (yes/no)	1.28	0.29–5.67	0.75
Tumor size (mm; continuous variable)	1.25	1.05–1.48	0.01
Hyperintensity on T2WI (yes/no)	6.79	2.15–21.46	0.001
Past history of HCC (yes/no)	1.29	0.50–3.34	0.61
SVR by DAAs (yes/no)	0.31	0.11–0.89	0.03
ALT (IU/L; continuous value)	1.01	1.00–1.02	0.02
Albumin (g/dL; continuous value)	1.80	0.66–4.88	0.25
Total bilirubin (mg/dL; continuous value)	2.62	0.83–8.25	0.10
PT (%; continuous value)	1.01	0.97–1.05	0.52
Alpha-fetoprotein (ng/mL; continuous value)	1.00	1.00–1.00	0.51
<i>Multivariate analysis</i>			
Sex (male)	2.14	0.77–5.90	0.14
Tumor size (mm)	1.14	0.95–1.36	0.16
Hyperintensity on T2WI (yes)	14.70	3.83–56.45	<0.001
SVR by DAAs (yes)	0.29	0.09–0.96	0.04
Alanine transaminase (IU/L)	1.01	1.00–1.02	0.17

p values were determined by Cox regression hazard analysis. Bold values are significant. ALT, alanine transaminase; CI, confidence interval; DAAs, direct-antiviral agents; HCC, hepatocellular carcinoma; HR, hazards ratio; SVR, sustained virologic response; T2WI, T2-weighted images.

Fig. 5. Cumulative rates for progression of an HBP-hypointense nodule without APHE to hypervascularization according to whether or not there was a past history of HCC.

Fig. 6. Representative images of an HBP-hypointense nodule without APHE on Gd-EOB-DTPA-enhanced MRI at the time of the initial diagnosis (upper panels) and 13 months later (lower panels). This nodule disappeared after achievement of an SVR.

detected in the non-SVR cohort (Table 2). Three of these nodules were diagnosed on dynamic CT as well as on Gd-EOB-DTPA-enhanced MRI before the initiation of DAA therapy. The median interval between the initial diagnosis of HHN without APHE on Gd-EOB-DTPA-enhanced MR images and their disappearance was 15 months (range 4–28). The characteristics of the nodules that disappeared are shown in Table 4.

Table 4. The characteristics of the disappeared nodules after eradication of HCV by DAAs

Nodule No.	Monitoring period after initial MRI, months	Nodule size, mm	T2WI	DWI	ER
1	16.7	10	Isointense	Isointense	0.93
2	13.5	8	Isointense	Isointense	3.61
3	28.3	6	Isointense	Isointense	1.41
4	11.1	6	Isointense	Isointense	1.78
5	4.2	7	Isointense	Isointense	1.88
6	12.8	8	Isointense	Isointense	1.49

DWI, diffusion-weighted imaging; MRI, magnetic resonance imaging; T2WI, T2-weighted imaging; ER, enhancement ratio.

Fig. 7. Correlation between the ER and the outcome of HBP-hypointense nodules without APHE in the SVR cohort. The ER was significantly higher for the hypovascular hypointense nodules that disappeared after achievement of an SVR than for those that progressed to hypervascular HCC ($p = 0.002$) or remained unchanged ($p = 0.019$); ° and * represent outliers.

Correlation between ER and Change of the Nodules on Imaging

To clarify the baseline radiological characteristics of HHNs without APHE that progressed to HCC on the initial screening images, we evaluated each ER for 65 HHNs without APHE in the SVR cohort. We found a correlation between the ER value at the initial diagnosis and the risk of HHNs without APHE progressing to HCC (Fig. 7). The mean ER was 0.59 ± 0.39 for HHNs without APHE that progressed to HCC and 1.15 ± 0.55 for those that did not. The mean ER for the HHNs without APHE that disappeared after the achievement of an SVR was 1.85 ± 0.93 . The ER was significantly higher in HHNs without APHE that disappeared than in those that progressed to HCC ($p = 0.002$) and those that remained unchanged ($p = 0.019$). We also evaluated each Delta T1 for HHN without APHE in the SVR cohort. Delta T1 ([post T1 value – pre T1 value]) was 127 ± 52 for HHNs without APHE that progressed to HCC, and 224 ± 123 for those that did not. For HHNs without APHE that disappeared after the achievement of SVR, Delta T1 was 227 ± 71 . Delta T1 was significantly lower in HHNs without APHE that progressed to HCC than in those that disappeared ($p = 0.014$) and those that remained unchanged ($p = 0.045$; data not shown).

Discussion

This study demonstrated that eradication of HCV with DAA therapy decreases the risk of progression of HHNs without APHE to HCC. Furthermore, our data indicate that the process of hepatocarcinogenesis, which is generally accompanied by loss of Gd-EOB-DTPA uptake capacity, can be altered by eradication of HCV at the HHN without APHE stage.

We found that achievement of SVR with DAA therapy may suppress the development of HCC (i.e., the hypervascularization of HHNs without APHE) in patients with a past history of HCC. Recently, Toyoda et al. [28] reported that eradication of HCV by DAA therapy had no influence on the development of HCC in patients with no past history of HCC. The discrepancy between the findings of our study and those reported by Toyoda et al. [28] may be explained by the high frequency of a past history of HCC in our cohort. Indeed, when we excluded those patients with a past history of HCC, we could not find an effect of SVR on prevention of hypervascularization in HHNs without APHE. These data suggest that development of HCC from HHNs without APHE may be less likely in patients without a past history of HCC, which might make the effect of an SVR on HHN without APHE less clear. We tried to evaluate the outcome of HHNs without APHE after IFN-based treatment but could not do so because achievement of SVR after IFN-based treatment was rare.

HCV is considered to increase the risk of progression to HCC by promoting inflammation, regeneration, and fibrosis of the infected liver [8, 29]. These adverse events accelerate hepatocyte turnover, which increases the risk of replication errors and DNA damage. Furthermore, numerous studies have demonstrated that HCV proteins, including HCV core, NS3, and NS5A, disrupt host signaling pathways regulating the cell cycle, cell growth, the stress response in the endoplasmic reticulum, steatosis, and response to DNA damage [30]. Our data suggest that about 10% of HHNs without APHE might revert to normal hepatocyte-like cell phenotypes in terms of OATP1B3 transporter activity. At least half of these HHNs without APHE were not only diagnosed by Gd-EOB-DTPA-enhanced MRI but also by dynamic CT, indicating that HHNs without APHE evaluated by Gd-EOB-DTPA-enhanced MRI had a characteristic reduction of portal venous flow, which is a hallmark of early-stage radiological findings of HCC. It is plausible that the reduction of OATP1B3 activity in dysplastic nodules is, at least in part, related to disruption of the signaling pathways induced by HCV proteins, although the details of the mechanisms involved have yet to be clarified.

HHNs without APHE on Gd-EOB-DTPA-enhanced MR images are thought to correspond to early HCC or a dysplastic nodule, which are at high risk for progression to classical hypervascular HCC. A recent meta-analysis reported that the cumulative incidence rates for progression of these nodules to HCC were 18.3% at 1 year, 25.2% at 2 years, and 30.3% at 3 years [31], which is consistent with our findings. Our data further indicate that HHNs without APHE can be classified into two subtypes, namely high-risk HHNs without APHE that are hyperintense on T2WI images, and low-risk HHNs without APHE that are isointense or hypointense on T2WI images. The hyperintensity of liver nodules on T2WI is generally considered to reflect dilated intratumoral sinusoids in HCC [32]. Our data indicate that achievement of SVR with DAA therapy prevents the progression of low-risk HHNs without APHE to HCC.

The expression levels of the OATP 1B3 protein or *OATP1B3* in the tumor and the ER on HBP images are generally decreased as the histology progresses from low-grade to high-grade dysplastic nodules, to early HCC, and to moderate HCC [25]. Furthermore, another recent study reported that hepatocyte nuclear factor 4 α (HNF4 α), a master regulator of hepatocyte function, and β -catenin, the main gatekeeper of metabolic zonation, coregulate expression of OATP1B3 and determine the ER on Gd-EOB-DTPA-enhanced MR images, which correlates with the histological differentiation of HCC [33]. HNF4 α is known to play

a role in the development of the liver, to control the function of hepatocytes, and to act as a tumor suppressor gene [34–36]. In addition, it has been reported that HCV infection suppresses the expression of HNF4 α at both the transcriptional and translational levels, and might be responsible for the mechanisms of HCV-related carcinogenesis in the liver [37]. We speculate that recovery of HNF4 α function after eradication of HCV by DAA therapy may help to suppress the multistep process of carcinogenesis and decrease the risk of progression of dysplastic nodules to HCC, although further studies are necessary to confirm this hypothesis.

In our study, disappearance of HHNs without APHE occurred in only the SVR cohort. Although we used 1.5- or 3-T scanners, quantitative indices of hepatic enhancement were similar to those in a previous report [38]. Given that the same MRI scanner and image acquisition conditions were used in the SVR and non-SVR cohorts, these data suggest that eradication of HCV might alter the characteristics of HHNs without APHE with activation of OATP1B3. Although these nodules were not examined histologically, they had a significantly higher ER than other nodules [26]. Well-differentiated HCC shows relatively high Gd-EOB-DTPA uptake with expression of HNF4 α ; therefore, eradication of HCV might improve expression of OATP1B3 via reactivation of HNF4 α , resulting in disappearance of HHNs without APHE. However, HHNs without APHE with a lower ER, those that were larger, and those that were hyperintense on T2WI were more likely to progress to HCC in our study. These nodules should be observed carefully even after achievement of an SVR.

Our study has several limitations. The first is its retrospective observational design and the variable follow-up protocols implemented after DAA therapy. Secondly, there was a degree of selection bias as a result of EOB-MRI being more likely to be performed in high-risk patients with HHNs without APHE, such as those with advanced liver fibrosis or a past history of HCC. Furthermore, the sample size might have been too small for a robust multivariate statistical analysis. A clustering effect resulting from the various treatment options (DAA therapy in the SVR group and IFN-based treatment or no treatment in the non-SVR group) might also have affected the results of our statistical analysis. Therefore, the results of this study should be interpreted carefully. Moreover, the nodules were not subjected to histological evaluation. However, it is not realistic to evaluate all HHNs without APHE by biopsy in routine clinical practice, so we evaluated the nodules using the ER, which has been reported to correlate with the tumor differentiation status [26]. Multicenter prospective studies designed to overcome these limitations are needed in the future. In conclusion, eradication of HCV by DAA therapy could reduce the hypervascularization rate of HHNs without APHE, and some of these nodules disappeared.

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Statement of Ethics

This study was conducted in accordance with the mandates of the Helsinki Declaration and the guidelines of the ethics committee of Kanazawa University Hospital.

Disclosure Statement

The authors have no conflicts of interest.

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Author Contributions

Y.Sh. performed acquisition and interpretation of clinical data, statistical analysis, and prepared the initial draft. K.A. and Tar.Y. designed the concept of the study and helped the preparation of the manuscript. Tat.Y., T.S., K.Ka., K.Ki., Y.Sa., E.M., and M.H. contributed to the recruitment of the patients. A.K., K.Ko., and S.Ko. performed the imaging analysis. S.Ka. supervised the study. All authors approved the final version of the manuscript.

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