**The Impact of HCV Eradication by Direct-acting Antivirals on the Transition of Precancerous Hepatic Nodules to HCC: a Prospective Observational Study**

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**List of Abbreviations**

DAA, direct-acting antiviral; Gd-EOB-DTPA, gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; MRI, magnetic resonance imaging; NHHN, non-hypervascular hypointense nodule; ROC, receiver operating characteristic; SVR, sustained virologic response.

**Conflict of interest**

Hidenori Toyoda reports lecturer fees from AbbVie and Bristol-Myers Squibb. Takashi Kumada report lecturer fees from Gilead Sciences and Bristol-Myers Squibb.

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**Abstract**

*Background & Aims*: It remains controversial whether the eradication of hepatitis C virus (HCV) by interferon (IFN)-free anti-HCV therapy using direct-acting antivirals (DAAs) suppresses or promotes hepatocellular carcinoma (HCC) development. We investigated the influence of HCV eradication by DAA therapy on HCC development, by observing changes of non-hypervascular hypointense nodules (NHHNs) by gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging (EOB-MRI). *Methods*: A total of 401 patients treated with DAA therapy who did not have a history of HCC were enrolled in this prospective cohort study. All patients underwent EOB-MRI prior to the start of DAA therapy and were followed up periodically after therapy. The progression of NHHNs detected at baseline to typical HCC, as indicated by hypervascularization and the incidence of newly emergent NHHNs, was analyzed. *Results*: In comparison of patients who achieved sustained virologic response (SVR) with propensity score-matched patients with persistent HCV infection, there was no difference in the incidence of hypervascularization of NHHNs to typical HCC among patients who had NHHNs at baseline. Among patients who did not have NHHNs at baseline, the incidence of the new emergence of NHHNs did not differ between study patients and propensity score–matched patients with persistent HCV infection. *Conclusions*: During a 2-year observation period after SVR, the eradication of HCV by IFN-free DAA therapy did not suppress or enhance HCC development. (UMIN000017020)

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**Key words**: hepatitis C virus; direct-acting antiviral therapy; hepatocellular carcinoma; non-hypervascular hypointense nodules

**Lay Summary**

During a 2-year observation period, the eradication of HCV by IFN-free DAA therapy did not influence hypervascularization of non-hypervascular hypointense nodules (NHHNs) detected by gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid–enhanced magnetic resonance imaging to typical HCC among patients who had NHHNs at baseline, and new emergence of NHHNs among patients who did not have NHHNs at baseline. No typical HCC occurred after the eradication of HCV when patients did not have NHHNs at baseline.

Word counts of the lay summary: 72 words

**Introduction**

 Hepatocellular carcinoma (HCC) [1,2] is one of the most important complication**s** of chronic HCV infection [3]. A paradigm shift in HCV treatment occurred with the introduction of interferon (IFN)-free therapy using direct acting antiviral agents (DAAs). These agents have an excellent safety profile and more than 90% of patients with HCV undergoing DAA therapy achieved sustained virologic response (SVR). Achievement of SVR with IFN-based anti-HCV therapy reportedly resulted in a marked decrease in the incidence of HCC [4-7]. However, the effectiveness of HCV eradication by DAA therapy in preventing the development of HCC after SVR remains controversial [8-16]. In particular, recent studies have suggested that HCV eradication by DAA therapy might enhance the risk of HCC development or, after resection, recurrence [8,9,17]. There is thus an urgent need to determine whether HCV eradication by DAA therapy will prevent or enhance HCC development in patients with chronic HCV infection.

 Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid–enhanced magnetic resonance imaging (EOB-MRI) is currently the most sensitive imaging modality to detect liver nodules [18]. Non-hypervascular hypointense nodules (NHHNs) are hepatic nodules that are detected during the hepatobiliary phase of EOB-MRI but lack enhancement during its arterial phase. The precise histological/pathological correlates of NHHNs, as detected by EOB-MRI, remain controversial. Where histological examination has been undertaken they are reported variously as ‘dysplastic nodules’ or ‘very early HCC’ [19]. In any case, NHHNs would not fit a clinical diagnosis of HCC according to current AASLD/EASL guidelines and, to this extent, we believe that such lesions most likely represent a ‘pre-clinical’ stage of HCC. Resected specimens examined after EOB-MRI evidence of hypervascularization are usually reported as well or moderately well-differentiated HCC and, as such represent a further step in the development of HCC [20,21]. Such lesions would be classified as HCC according to AASLD/EASL guidelines [22,23].

 In this prospective study we have applied this most sensitive method for the detection of preclinical HCC to examine the impact of HCV eradication on the transition of pre-clinical to clinical HCC. We have also examined the impact of HCV eradication on the emergence of new such lesions in patients without these nodules before therapy.

**Patients and Methods**

*Study Patients*

 Between September 2014 and May 2016, 561 patients with chronic-HCV hepatitis or compensated HCV-related cirrhosis were initiated on DAA therapy. Of these, 470 underwent EOB-MRI within 2 weeks of starting therapy. Ninety-one patients did not undergo EOB-MRI for the following reasons; implantation of metal materials, including pacemakers, bolts for bone fractures, etc., in 29 patients; renal dysfunction in 47 patients; claustrophobia in 9 patients; and patient refusal in 6 patients. Sixty-nine of the 470 patients who underwent pretreatment EOB-MRI had a history of curative HCC treatment. Because this study focused on the development of de novo HCC rather than recurrence, these patients were excluded. The remaining 401 patients were enrolled in this study (Figure 1). No patient with decompensated liver cirrhosis was included in these 401 patients because Japanese Medical Insurance does not allow anti-HCV therapy with DAAs in patients with decompensation. Also, no patients with coinfection with hepatitis B virus or HIV were included.

 After DAA therapy, patients underwent routine ultrasound surveillance for HCC every 3 to 6 months according to Japanese guideline [24]. EOB-MRI examination was repeated every 6 to 9 months in patients in whom NHHNs had been detected at baseline. In patients without NHHNs at baseline, EOB-MRI examination was repeated every 6 to 12 months in addition to routine surveillance ultrasonography.

 This prospective study was conducted after approval by the institutional review board of Ogaki Municipal Hospital and was carried out in compliance with the Helsinki Declaration. Written informed consent was obtained from all participating patients.

*Pretreatment Imaging Examination of Liver Nodules using* *EOB–MRI and Confirmation of Non-hypervascular Hypointense Nodules*

 Patients underwent EOB-MRI within 2 weeks of the start of anti-HCV therapy using a 3.0 -T whole -body MRI system (Discovery MR 750W 3.0T; GE Healthcare Japan, Tokyo, Japan). All imaging findings were independently evaluated by a radiologist (Y.S.) and 2 hepatologists (H.T. and T.T.) who were blinded to the clinical data. Existence of NHHNs was evaluated by comparing the dynamic arterial phase and the hepatobiliary phase of EOB-MRI images. When liver nodules that were not typical for NHHNs were noted, other imaging examinations including contrast-enhanced ultrasonography or contrast-enhanced computed tomography were conducted as appropriate. If imaging assessments between the reviewers were discordant, consensus was achieved through discussion.

*Control Patients*

 To compare the incidence of hypervascularization of NHHNs and the emergence of new NHHNs between patients in whom HCV was eradicated by DAA therapy and patients with persistent HCV infection, the rates of hypervascularization and new emergence were investigated in patients with persistent HCV infection who had undergone EOB-MRI. A total of 217 patients with chronic HCV infection who did not have a history of HCC underwent EOB-MRI between 2010 and 2015. Among these patients, NHHNs were detected in 59 patients (27.2%). After propensity score matching, the incidence of hypervascularization of these nodules was analyzed and compared with that in patients in whom HCV was eradicated. Similarly, among 158 patients in whom NHHNs were not detected at baseline, the incidence of new NHHNs was analyzed and compared with that of patients in whom HCV was eradicated after propensity score matching (Figure 2).

*Statistical Analysis*

 Differences in percentages between groups were analyzed using the chi-square test. Quantitative values were compared using the Mann-Whitney U test. Univariate and multivariate analyses were performed for factors associated with the presence of NHHNs on Gd-EOB-DTPA–enhanced MRI at baseline. Only factors that showed a statistically significant association (*P*<0.05) with the presence of NHHNs by univariate analysis were included in the multivariate analysis; these factors were age, sex, baseline platelet count, baseline ALT, ALB, T-Bil, and AFP.

 The date of the baseline MRI study was defined as time zero for calculations of the incidence of hypervascularization of NHHNs or new emergence of such nodules. In the analysis of the incidence of hypervascularization of NHHNs among patients with NHHNs at baseline, those who demonstrated hypervascularization of these nodules were not censored, while those with no hypervascularization were censored. In the analysis of the emergence of new NHHNs among patients without NHHNs at baseline, those in whom NHHNs newly emerged were not censored, while those who did not develop NHHNs were censored. Statistical analysis was performed using JMP statistical software, version 11.0.0 (Macintosh version; SAS Institute, Cary, NC). All *P* values were derived from two-tailed tests, with *P*<0.05 accepted as statistically significant.

 For propensity score matching to compare the incidence of hypervascularization of baseline NHHNs by Gd-EOB-DTPA–enhanced MRI and the incidence of new NHHNs between patients in whom HCV was eradicated by DAA therapy and those with persistent HCV infection, we conducted one-to-one pairing of patients based on age, gender, ALT, platelet counts, and cirrhosis with propensity scores matched to two decimal places. The discriminative ability of the propensity score model was assessed using an area under the receiver operating characteristic (ROC) curve. Calibration was assessed using the Hosmer–Lemeshow goodness-of-fit test. Statistical analysis was performed using SPSS, version 18.0 (IBM, Tokyo, Japan).

**Results**

*Baseline Characteristics of Study Patients*

 Table 1 shows the characteristics of the study patients. Presence of cirrhosis was defined clinically by presence of esophageal/gastric varices, collateral veins due to portal hypertension, or splenomegaly and was observed in 112 patients (27.9%). NHHNs were detected in 35 patients (8.7%) by EOB-MRI before DAA therapy. The size of NHHNs was 8.5 (3-19) mm. SVR was achieved in 383 patients (95.5%) by DAA therapy. NHHNs were detected at baseline in 33 of 383 patients (8.6%) who achieved SVR and 2 of 18 patients (11.1%) who failed to achieve SVR.

*Hypervascularization of Baseline NHHNs and Emergence of New NHHNs after the Eradication of HCV*

 Patients were followed up for 12.7 ± 4.6 months (median, 13.6 months) after SVR. No patients were lost to follow-up. Typical hypervascular HCC developed in 6 patients after DAA therapy: 5 patients who achieved SVR and one patient who failed to achieve SVR. HCC developed in 3 of 190 patients (1.58%) who underwent daclatasvir + asunaprevir for 24 weeks (including one patient with failure of SVR), one of 96 patients (1.04%) who underwent sofosbuvir + ledipasvir for 12 weeks, and 2 of 115 patients (1.74%) who underwent sofosbuvir + ribavirin for 12 weeks.

 Among 383 patients who achieved SVR, 33 had NHHNs detected before DAA therapy. Hypervascularization of NHHNs was observed in 5 of 33 patients (15.2%, Supplementary figure 1, Table 2a). All of these liver nodules were confirmed as typical hypervascular HCC by dynamic enhanced computed tomography and angiography after EOB-MRI. All patients underwent hepatic resection, and HCC was confirmed histologically based on the resected specimens. The resected HCCs were well-differentiated in 3 patients and were moderately differentiated in the remaining 2 patients. All but one had histologically confirmed cirrhosis at resection. The sizes of NHHNs at baseline were 3 to 16 mm, and increased after hypervascularization except in one patient. In another patient, one of 2 NHHNs showed hypervascularization.

 Among the 350 patients in whom NHHNs were not detected at baseline and who achieved SVR, 8 patients (2.3%) demonstrated the new development of such nodules (Supplementary figure 2). No hypervascular typical HCCs were detected in these 350 patients.

*Hypervascularization Rates of NHHNs in Patients with HCV Eradication versus Patients with Persistent HCV Infection: Propensity Score-Matched Comparison Among Patients with NHHNs at Baseline*

 Among patients who had NHHNs at baseline, propensity score matching was used to compare the incidence of hypervascularization of NHHNs in patients who achieved SVR with that in patients with persistent HCV infection. Thirty-three patients with persistent HCV infection were selected as matched controls. As shown in Supplementary Table 1, there were no significant differences in the characteristics of patients who achieved SVR and those who did not. In the control patients, typical hypervascular HCC developed in 8 patients in the observation period (Table 2b).

 Figure 3 and supplementary figure 3 compare the incidence and time interval of the transition from NHHNs to typical hypervascular HCC in patients who achieved SVR and those with persistent HCV infection. Follow-up EOB-MRI studies began at approximately 6 months after baseline. There was no significant difference in the incidence of hypervascularization at 12, 18, 24 months between those who did achieve SVR (11.8%, 24.2% and 25.2%) and those with persistent HCV infection (9.1%, 15.2%, and 24.9%) respectively (*P*=0.6170).

*Emergence of NHHNs in Patients with HCV Eradication and Patients with Persistent HCV infection: Propensity Score-Matched Comparison Among Patients without NHHNs at Baseline*

 Among patients who did not have NHHNs at baseline, propensity score matching was used to compare the incidence of the emergence of new NHHNs in patients who achieved SVR with that in patients with persistent HCV infection (control subjects). One hundred and thirty-nine patients were compared with matched controls. As shown in Supplementary Table 2, there were no significant differences in the characteristics of patients who achieved SVR and those of the controls.

 Figure 4 compares the incidence of the emergence of NHHNs between patients who achieved SVR and those with persistent HCV infection. Follow-up EOB-MRI studies began at 6 months after baseline. The incidences of the emergence of these nodules at 12, 18, 24 months were 3.4%, 3.4%, and 10.3% in patients with SVR, and 5.2%, 5.2%, and 8.9% in patients with persistent HCV infection, respectively. The incidences did not differ between two groups (P=0.7282).

**Discussion**

 In the present study, patients with NHHNs at baseline were prospectively observed for up to 2 years and during this period, the incidences of hypervascularization of these nodules, i.e., transition to typical HCC, were similar between patients with HCV eradication and those with persistent HCV infection. Also, when considering only patients without NHHNs at baseline, both groups showed a similar incidence of the emergence of these nodules. Thus, the likelihood of developing HCC did not change during the 2-year observation period after the eradication of HCV. We found no evidence that SVR either suppressed or promoted HCC development.

 Another important finding was that no patient experienced the direct emergence of hypervascular HCC after SVR whereas some patients with baseline NHHNs showed transition to hypervascular HCC. This suggests that, in most cases, typical hypervascular HCC develops as the hypervascularization of NHHNs, and rapid emergence of hypervascular HCC would not occur after SVR.

 Although considerable controversy surrounds the topic, evidence both in support of, and against, the preventive effect of SVR on HCC development has, until now, relied on analysis of retrospective data. The main strength of our current paper is that it prospectively tests the hypothesis that HCV eradication influences HCC development and found no evidence in support of such a hypothesis.

 There are however, also limitations to this study. The period of observation was short. The age of patients with chronic hepatitis C who are candidates for anti-HCV therapy with DAAs is higher in Japan than in Western countries, and the influence of HCV eradication on HCC development may differ among regions. Another limitation is that the study population did not include patients with decompensated cirrhosis, for whom DAA therapy is not allowed in Japan. In addition, the control patients, who had persistent HCV infection, were not randomized with the patients who achieved SVR but with the current availability of effective (DAA) antiviral therapy such randomization would be neither practical nor ethical. Nonetheless, the 2 groups’ backgrounds were balanced using propensity score matching. Although NHHNs at baseline might have been a mixture of precancerous dysplastic nodules and non-hypervascular very-early stage HCC in both groups, the mixture could also be balanced by propensity score matching. Finally, the present study focused on patients without a history of HCC. Therefore, the effects of HCV eradication on the recurrence of HCC in patients who had completed HCC treatment may differ from those demonstrated in this study and should be investigated further. In particular, there are two distinct patterns in cases of the recurrence of HCC after SVR: hypervascularization of NHHNs at baseline and the direct emergence of hypervascular typical HCC without baseline NHHNs. These two patterns may reflect multicentric recurrence and intrahepatic metastasis [25]. The effect of HCV eradication on the recurrences of HCC should be analyzed being categorized based on the pattern of recurrences.

 In conclusion, amongst patients without a history of HCC, we found no evidence that eradication of HCV by DAA therapy influenced HCC development (as shown by hypervascularization of NHHNs) or the new emergence of NHHNs.

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**Figure Legends**

Figure 1. Schematic flowchart of the enrollment of study patients.

Figure 2. Schematic flowchart comparing propensity score-matched patients with and without non-hypervascular hypointense nodules at baseline. These patients were derived from 2 groups: patients with HCV eradication (cases) and those with persistent HCV infection (controls)

Figure 3. Hypervascularization rates of non-hypervascular hypointense nodules in patients with HCV eradication by DAA therapy versus patients with persistent HCV infection: propensity score-matched comparison among patients with non-hypervascular hypointense nodules at baseline. No difference was observed between the 2 groups.

Horizontal axis, period after baseline (months). Red line, patients with HCV eradication. Blue line, patients with persistent HCV infection.

Figure 4. Rates of the emergence of non-hypervascular hypointense nodules in patients with HCV eradication by DAA therapy and patients with persistent HCV infection: propensity score-matched comparison among patients without non-hypervascular hypointense nodules at baseline. No difference was observed between the 2 groups.

Horizontal axis, period after baseline (months). Red line, patients with HCV eradication. Blue line, patients with persistent HCV infection.

Supplementary figure 1. Hypervascularization of baseline non-hypervascular hypointense nodules (transition to typical HCC) after the eradication of HCV by DAA therapy.

A) Before the start of DAA therapy (baseline), B) at the end of the therapy (24 weeks after the start of therapy), C) 48 weeks after the end of therapy (72 weeks after the start of therapy). This patient had a non-hypervascular hypointense nodule at baseline (red arrow) and underwent a 24-week regimen of daclatasvir and asunaprevir, achieving SVR. A mild increase in the size of the non-hypervascular hypointense nodule was observed, as well as enhancement during arterial phase of EOB-MRI (white arrow). Hypervascularization occurred at 48 weeks after the end of DAA therapy. The patient underwent hepatic resection and HCC was confirmed pathologically.

Supplementary figure 2. Emergence of non-hypervascular hypointense nodules in patients with HCV eradication by DAA therapy.

A) Before the start of DAA therapy (baseline), B) 48 weeks after the end of therapy (60 weeks after the start of therapy). This patient did not have a non-hypervascular hypointense nodule at baseline and underwent a 12-week regimen of ledipasvir and sofosbuvir, achieving SVR. A new non-hypervascular hypointense nodule was observed at 48 weeks after the end of DAA therapy (red arrowhead).

Supplementary figure 3. Time interval of baseline and follow-up examinations by EOB–MRI, period of DAA therapy (case patients) and development of HCC in patients with non-hypervascular hypointense nodules at baseline.

A) Patients with HCV eradication by DAA therapy (cases)

B) Patients with persistent HCV infection (controls)

In each patient, the vertical bars indicate the examinations by EOB–MRI and blue areas in case patients indicate DAA treatment periods. Red dots indicate the hypervascularization of NHHNs, being diagnosed as HCC.