**Viral eradication reduces multistep hepatocarcinogenesis in patients with chronic hepatitis C virus infection: A prospective serial follow-up MRI study.**

Takashi Kumada1), Hidenori Toyoda2), Satoshi Yasuda2), Toshifumi Tada2), Yasuhiro Sone3), Sadanobu Ogawa4), Kenji Takeshima4), Junko Tanaka5), Kazuaki Chayama6)

1) Department of Nursing, Faculty of Nursing, Gifu Kyoritsu University, Ogaki, Gifu, Japan

2) Department of Gastroenterology and Hepatology, Ogaki Municipal Hospital, Ogaki, Gifu, Japan

3) Department of Radiology, Ogaki Municipal Hospital, Ogaki, Gifu, Japan

4) Department of Imaging Diagnosis, Ogaki Municipal Hospital, Ogaki, Gifu, Japan

5) Department of Epidemiology, Infectious Disease Control, and Prevention, Hiroshima University Institute of Biomedical and Health Sciences, Hiroshima, Japan

6) Department of Gastroenterology and Metabolism, Institute of Biomedical and Health Sciences, Hiroshima University Hospital, Hiroshima, Japan

Short running title: Viral eradication and multistep hepatocarinogenesis

Lay Summary

With the advent of gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid–enhanced magnetic resonance imaging (Gd-EOB-MRI), differentiation of two types of hepatocarcinogenesis, “multistep” and “de novo”, has become possible. We analyzed whether hepatitis C virus (HCV) eradication suppressed hepatocarcinogenesis using serial Gd-EOB-MRI. The eradication of HCV by direct-acting antiviral therapy reduces multistep hepatocarcinogenesis and de novo hepatocarcinogenesis was observed in patients with high *Lens culinaris* agglutinin–reactive fraction of alpha-fetoprotein values.

**\*Corresponding author: Takashi Kumada, MD**

Department of Nursing, Faculty of Nursing, Gifu Kyoritsu University

5-50, Kitagata-cho, Ogaki-shi, Gifu-ken, 503-8550, JAPAN

TEL: +81-584-77-3512 FAX: +81-584-77-3510

E-mail: takashi.kumada@gmail.com

E-mail address of coauthors

Hidenori Toyoda: tkumada@he.mirai.ne.jp

Satoshi Yasuda: satoshi.yasuda.1982@gmail.com

Toshifumi Tada: tadat0627@gmail.com

Yasuhiro Sone: [soney-kts@theia.ocn.ne.jp](mailto:soney-kts@theia.ocn.ne.jp)

Sadanobu Ogawa: sada4577@yahoo.ne.jp

Kenji Takeshima: k-takeshima@octn.jp

Junko Tanaka: jun-tanaka@hiroshima-u.ac.jp

Kazuaki Chayama: chayama@mba.ocn.ne.jp

Word count: 3,054

Number of tables: 3

Number of figures: 7

**List of abbreviations**: HCC, hepatocellular carcinoma; ALBI, albumin–bilirubin; DAA, direct-acting antiviral; Gd-EOB-MRI, Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid–enhanced magnetic resonance imaging; HBP, hepatobiliary biliary phase; CT, computed tomography; NHHN, nonhypervascular hypointense nodule; HCV, hepatitis C virus; SVR, sustained virological response; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, alpha-fetoprotein; AFP-L3%, *Lens culinaris* agglutinin–reactive fraction of alpha-fetoprotein; DCP, des-carboxy prothrombin; US, ultrasonography

Conflicts of interest: Takashi Kumada (Gilead Sciences, AbbVie, Ezai), Hidenori Toyoda (Gilead Sciences, AbbVie).

Financial support: This work was supported by Health and Labour Sciences Research Grants (Research on Hepatitis) from the Ministry of Health, Labour and Welfare of Japan.

**Abstract**

**Background and Aims**: With the advent of direct-acting antiviral (DAA) drugs, sustained virological response (SVR) can be easily achieved in almost all patients with hepatitis C virus (HCV) infection. We conducted a prospective study using gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid–enhanced magnetic resonance imaging (Gd-EOB-MRI) to determine whether the eradication of HCV suppressed hepatocarcinogenesis.

**Methods and Materials**: A total of 719 patients were enrolled, including 509 patients in the “DAA group” who achieved SVR by DAAs and 210 patients in the “Non-DAA group” who did not receive DAAs. Hepatocarcinogenesis was classified into two types, “multistep” and “de novo”. Factors associated with each type were analyzed by Fine and Gray proportional hazards models.

**Results:** Of 67 patients with hepatocellular carcinoma, multistep hepatocarcinogenesis occurred in 58 patients (86.6%) and de novo hepatocarcinogenesis occurred in nine patients (13.4%). Factors associated with multistep hepatocarcinogenesis were male gender (hazard ratio [HR], 2.543; 95% confidence interval [CI], 1.645–4.413; p=0.0070) and DAA therapy (HR, 0.4053; 95%CI, 0.2103–0.7812; p=0.0009). By contrast, factors associated with de novo hepatocarcinogenesis were *Lens culinaris* agglutinin–reactive fraction of alpha-fetoprotein (AFP-L3%) ≥ 5% (HR, 25.0; 95%CI, 1.361–459.2; P=0.0300) and albumin–bilirubin grade 2 or 3 (HR, 141,700; 95%CI, 50,250–399,600; P<0.0001).

**Conclusion:** The eradication of HCV by DAA therapy reduces multistep hepatocarcinogenesis. De novo hepatocarcinogenesis was observed in patients with high AFP-L3% values.

Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of death from cancer worldwide [1]. It is well known that hepatitis C virus (HCV) infection is one of the main causes of HCC. In the current era of therapy with direct-acting antiviral (DAA) drugs, sustained virological response (SVR) can be easily achieved in ≥ 90% of patients with HCV infection. The benefits of SVR are well recognized, and include reduced rates of not only HCC but also liver complications, mortality, and extrahepatic and systemic complications [2]. However, the occurrence of HCC cannot be completely suppressed even if the HCV is eradicated and continued surveillance is still necessary. Long-term follow-up study of cirrhotic patients who achieved SVR with interferon-based therapy reported an approximately 75% reduction in HCC risk [3]. Many recent studies, despite short observation periods, showed that DAA therapy reduced the risk of HCC occurrence as effectively as interferon-based therapy [4, 5, 6].

Recently, magnetic resonance imaging (MRI) using gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) has been increasingly used to evaluate hepatic focal lesions, including HCC [7]. Gd-EOB-DTPA is unique in that approximately 50% of the administered dose is taken up by hepatocytes via the organic anion–transporting polypeptide 1B3 (OATP1B3), then excreted into the bile ducts via the multidrug resistance protein 2 (MRP2) [8]. Therefore, Gd-EOB-DTPA–enhanced MRI (Gd-EOB-MRI) can provide not only information about the vascular phase, but also information regarding the hepatobiliary biliary phase (HBP) [9]. Recent guidelines have recommended Gd-EOB-MRI as an effective diagnostic tool for the noninvasive diagnosis of HCC [10, 11, 12]. Multiple studies have compared the diagnostic performance of multiphasic computed tomography (CT) with that of Gd-EOB-MRI; all showed that Gd-EOB-MRI had higher sensitivity and similar specificity, the difference being significant in patients with small lesions [13, 14, 15, 16]. Furthermore, nonhypervascular hypointense nodules (NHHNs) visible during the HBP of Gd-EOB-MRI have a higher risk of progression to typical HCC than iso- or hyperintense nodules [17, 18].

The purpose of this study was to use serial Gd-EOB-MRI to reveal whether HCV eradication suppressed hepatocarcinogenesis. This is the first prospective study to evaluate hepatocarcinogenesis after SVR using Gd-EOB-MRI.

**Materials and Methods**

**Study Patients and Surveillance**

Between February 2008 and December 2018, 1083 consecutive patients with HCV-related chronic hepatitis or compensated cirrhosis underwent Gd-EOB-MRI. The present study cohort had the following enrollment criteria: (1) positivity for anti-HCV antibodies by second- or third-generation enzyme-linked immunosorbent assay and detectable HCV RNA for at least 6 months; (2) no evidence of positivity for hepatitis B surface antigen; and (3) exclusion of other causes of chronic hepatitis (i.e., alcohol consumption lower than 80 g/day, no history of hepatotoxic drug use, and negative tests for autoimmune hepatitis, primary biliary cholangitis, hemochromatosis, and Wilson’s disease). Of the 1083 enrolled patients, 606 were administered DAAs. One hundred and two patients did not undergo Gd-EOB-MRI within 3 months before starting DAA therapy. Ten patients did not achieve SVR and 87 patients had preexisting HCC at the time DAA therapy was initiated. The remaining 509 patients were enrolled as the “DAA group.” Four hundred and seventy-seven patients did not receive DAAs during the follow-up period. Of these, at the time of the first Gd-EOB-MRI, 159 patients had preexisting HCC and 108 patients had achieved SVR by interferon-based therapy.

This section is confusing! Why, if this was a prospective study, did 102 patients not get ‘Gd-EOB-MRI within 3 months before starting DAA therapy’? why include patients with ‘preexisting HCC’? Isn’t it likely that the progression of dysplastic nodules will be different in livers already harboring HCC?

***The remaining 210 patients were enrolled as the “Non-DAA group” (Figure 1)***.

This is the major weakness of the paper – you don’t say how it was decided to give or not give the DAAs

All patients underwent routine ultrasound and evaluation of tumor markers (alpha-fetoprotein [AFP], *Lens culinaris* agglutinin–reactive fraction of AFP [AFP-L3%], and des-carboxy prothrombin [DCP]) for HCC surveillance every 3 to 6 months according to the Japanese HCC guidelines [19]. Gd-EOB-MRI examination was repeated every 6 to 9 months in patients in whom NHHNs were detected during the HPB of Gd-EOB-MRI at baseline or during the follow-up period. In patients without NHHNs at baseline, Gd-EOB-MRI examination was repeated every 12 to 24 months, in addition to routine surveillance ultrasonography and tumor marker evaluation. The FIB-4 index was calculated at the start of follow-up by the following formula: FIB-4 = aspartate aminotransferase (AST) concentration (IU/L) × age (years) / (platelet count [109/L] × alanine aminotransferase [ALT] concentration1/2 [IU/L]) [20]. We used previously published cut-off values for the FIB-4. Patients with a FIB-4 value <1.45 were classified having no or moderate fibrosis, while those with a FIB-4 value >3.25 were defined as having extensive fibrosis or cirrhosis [21]. The albumin–bilirubin (ALBI) grade was calculated using the following equation: linear predictor = (log10 bilirubin μmol/L × 0.66) + (albumin/L × −0.085) [22]. The continuous linear predictor was further categorized into three different grades for prognostic stratification purposes, as previously described: grade 1 (less than −2.60), grade 2 (between −2.60 and −1.39), and grade 3 (above −1.39) [22]. AFP, AFP-L3%, and DCP were all measured using the same serum sample from each patient. The measurements of all three biomarkers were performed using a microchip capillary electrophoresis and liquid-phase binding assay on a µTAS Wako i30 auto analyzer (Wako Pure Chemical Industries, Osaka, Japan) [23].

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 participants. This trial is registered with University Hospital Medical Information Network (UMIN) Center, number UMIN00001702.

**Imaging Methods and Image Analysis**

Before March 2015, MRI was performed with a 1.5-T whole-body MRI system (Intera Achieva 1.5 T Nova; Philips Healthcare, Tokyo, Japan) with a phased-array body coil as the receiver coil. T1-weighted sequences were performed with a T1-weighted turbo field-echo in-phase and opposed-phase transverse sequence (opposed phase TE, 2.3; in-phase TE, 4.6; flip angle, 12°; matrix size, 256 × 512; scan percentage, 70; section thickness, 3.5 mm; intersection gap, 0 mm; field of view, 38 cm2). After IV injection of gadoxetate disodium (Primovist; Bayer Schering Pharma), a T1-weighted transverse gradient-echo sequence was performed (high-resolution isotropic volume examination with spectral pre-saturation and inversion recovery; TR/TE, 4/1.8; flip angle, 12°; matrix size, 256 × 512; scan percentage, 78.54; section thickness, 3.5 mm; intersection gap, 0 mm; field of view, 38 cm2). After April 2015, a 3.0-T whole-body MRI system was used (Discovery MR 750 W 3.0 T; GE Healthcare Japan, Tokyo, Japan). T1-weighted sequences were performed with a T1-weighted turbo field-echo in-phase and opposed-phase transverse sequence (opposed phase TE, 1.0; in-phase TE, 2.0; flip angle, 15°; matrix size, 256 × 224; phase FOV, 0.9; section thickness, 3.0 mm; intersection gap, 0 mm; field of view, 36 cm2). After intravenous (IV) injection of gadoxetate disodium, a T1-weighted transverse gradient-echo sequence was performed (LAVA-flex; a 3D, FSPGR imaging technology that generates water-only, fat-only, and in-phase and out-of-phase echoes in a single acquisition; TR/TE, 5/2; flip angle, 12°; matrix size, 224 × 320; phase FOV, 0.8; section thickness, 3.0 mm; intersection gap, 0 mm; field of view, 34 cm2). Gadoxetate disodium was administered intravenously as a bolus at a rate of 2 mL/s (0.1 mL/kg; maximal dose, 10 mL) through a cubital IV line (20–22 gauge) that was flushed with 20 mL of saline solution using a power injector (Sonic Shot; Nemoto Kyourindo).

One radiologist (Y.S., with 25 years of experience) and one gastroenterologist specializing in hepatology (T.K., with 30 years of experience) reviewed images to detect hypovascular nodules and to diagnose subsequent hypervascularization by consensus. A nodule was defined as hypovascular if its entirety showed lower signal intensity than the surrounding liver parenchyma during the arterial phase of dynamic imaging with all of the available modalities (intravenous contrast-enhanced CT, CT hepatic arteriography, and contrast-enhanced ultrasound) compared with the corresponding site on the unenhanced image. Arterial enhancement was assessed by means of visual inspection. In addition, the subjects were limited to those with round hypointense lesions during the HBP of Gd-EOB-MRI. Imaging procedures with all modalities were performed within 1 month of each other. Nodules were excluded if they were (a) less than 2 mm in diameter; or (b) considered to be suspicious for hemangiomas, cysts, or cystic tumors on the basis of other MR imaging sequences or modalities. Recent reports have suggested that hepatocellular nodules without hyperenhancement, detected as NHHNs during the HBP of Gd-EOB-MRI, are precursors of progressed HCC [17, 18]. The Hepatobiliary Agent Working Group of the Liver Reporting and Data System (LI-RADS) proposed the following standardized term for NHHNs: “HBP hypointense nodule without arterial phase hyperenhancement (APHE)” [24]. In this study, we used the shorter term “NHHNs” instead of “HBP hypointense nodule without APHE.”

In this analysis, hepatocarcinogenesis was classified into two types, “multistep” and “de novo” [25, 26]. Multistep hepatocarcinogenesis is defined as progression from NHHNs, which is equivalent to either dysplastic nodules (DNs) or early HCC, to hypervascular nodules such as advanced HCC (Figure 2). By contrast, de novo hepatocarcinogenesis is defined as the direct progression to hypervascular HCC without preexisting NHHNs (Figure 3).

**Statistical Analysis**

Continuous variables were expressed as medians (interquartile range). The Kruskal–Wallis test was used for continuous variables, and the Steel Dwass test was performed as post hoc testing when the Kruskal–Wallis test showed a significant difference. The chi-square test with Fisher’s exact test was used for categorical variables.

Actuarial analysis of cumulative occurrence for each type of hepatocarcinogenesis (multistep and de novo) was performed using the cumulative incidence with the competing risks method; differences were tested using the Gray test with Holm correction. For multivariate analysis, Fine and Gray proportional hazards models were used for the assessment of hazard ratios (HRs) for the occurrence rate [27].

Statistical significance was defined as p<0.05. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.

**Results**

**Comparison of characteristics of patients with no hepatocarcinogenesis (Group A), multistep hepatocarcinogenesis (Group B), and de novo hepatocarcinogenesis (Group C)**

This is the second big issue. The purpose of this study was ‘to use serial Gd-EOB-MRI to reveal whether HCV eradication suppressed hepatocarcinogenesis’ [Page 8]. So, you have to deal with this question first i.e. to compare A vs B+C.

Then you can talk about de novo and multistep carcinogenesis is a clearly separated section of the results. As it stands the message is not clear because you are discussing two separate issues - the impact o HCV eradication on hepatocarcinogenesis and the pathways to carcinogenesis – direct or de novo.

Hepatocarcinogenesis was observed in 67 of 719 (9.3%) patients during follow-up period. Multistep hepatocarcinogenesis occurred in 58 patients (86.6%) and de novo hepatocarcinogenesis occurred in nine patients (13.4%) (Figure 4). Among the latter, seven patients showed hypervascular liver nodules without preexisting NHHNs, and the remaining two patients demonstrated hypervascular nodules at a different site from the NHHNs. The incidence rates of multistep and de novo hepatocarcinogenesis were 11.2% and 2.0% at 3 years, respectively, and 21.2% and 3.6% at 5 years, respectively (Figure 5).

Table 1 shows the patient characteristics among the three groups at the start of follow-up. AST, ALT, and DCP were significantly higher in Group B than in Groups A and C (p<0.001). Total bilirubin, FIB-4, and AFP were significantly higher in Groups B and C than in Group A (p<0.001). Albumin and platelet counts were significantly lower in Groups B and C than in Group A (p<0.001). AFP-L3% was significantly higher in Group C than in Groups A and B (p<0.001).

**Factors associated with multistep hepatocarcinogenesis**

Table 2 shows the factors associated with multistep hepatocarcinogenesis according to Fine and Gray proportional hazards models. Factors that were analyzed were age, gender, therapy (non-DAA vs. DAA), FIB-4, ALBI grade, estimated glomerular filtration rate (eGFR), AFP, AFP-L3%, and DCP. Multivariate analysis showed that male gender (HR, 2.543; 95% confidence interval [CI], 1.645–4.413; p=0.0070) and DAA therapy (HR, 0.4053; 95%CI, 0.2103–0.7812; p=0.0009) were independently associated with multistep hepatocarcinogenesis. Figure 6 shows that the cumulative incidence rates of HCC with and without DAA therapy were 6.9% and 14.9% at 3 years, respectively, and 9.0% and 26.4% at 5 years, respectively, and there was a significant difference between the two groups (p=0.00281, Gray test).

**Factors associated with de novo hepatocarcinogenesis**

Table 3 shows the factors associated with de novo hepatocarcinogenesis according to Fine and Gray proportional hazards models. The analyzed factors were the same as those for multistep hepatocarcinogenesis. Multivariate analysis showed that AFP-L3 ≥ 5% (HR. 25.0; 95%CI, 1.361–459.2; p=0.0300) and ALBI grade 2 or 3 (HR, 141,700; 95%CI, 50,250–399,600; p<0.0001) were independently associated with de novo hepatocarcinogenesis. Figure 7 shows that the cumulative incidence rates of AFP-L3 ≥ 5% and AFP-L3 < 5% were 7.9% and 0.3% at 3 years, respectively, and 13.8% and 0.3% at 5 years, respectively, and there was a significant difference between the two groups (p<0.0001, Gray test).

Discussion

Two types of hepatocarcinogenesis, de novo and multistep, are now considered to cause HCC [25, 26]. De novo hepatocarcinogenesis results in the direct development of advanced HCC, whereas in multistep hepatocarcinogenesis a hepatocellular nodule progresses from a DN, to a DN with malignant foci, to a well-differentiated HCC nodule, and finally to classic HCC. In this study, multistep hepatocarcinogenesis accounted for 86.7% of HCC cases. With the advent of Gd-EOB-DTPA, the observation of multistep hepatocarcinogenesis has become easier. Gd-EOB-DTPA has unique pharmacohemodynamics, with contrast taken up by hepatocytes and excreted into the bile ducts. Narita et al. and Kitao et al. demonstrated a significant correlation between the expression of the uptake transporter OATP1B3 and signal intensity during the HBP in HCCs [29, 30]. The expression of OATP1B3 in hepatocellular nodules shows a significant positive correlation with the degree of tumor differentiation [31]. The enhancement ratio ([1/postcontrast T1 value − 1/precontrast T1 value] / [1/precontrastT1 value]) during HBP is a good indicator of tumor differentiation, except that moderately differentiated HCCs (6%) occasionally show a high enhancement ratio with increased OATP1B3 expression [31]. Gd-EOB-MRI has made it possible to detect high-grade DN or early HCC. In this study, NHHNs were detected in 101 patients at the first MRI and in 25 patients during the follow-up period. Clinically, almost all HCCs with metastases or vascular invasion are hypervascular relative to the surrounding liver. By contrast, high-grade DNs and early HCCs do not usually demonstrate any overt metastases or vascular invasion, and their treatment does not confer a survival benefit [32]. Therefore, we considered both entities to be clinically benign and did not perform any intervention. In fact, all patients received curative therapy after the diagnosis of overt hypervascular HCCs, as follows: 33 patients with hepatic resection, 25 patients with percutaneous radiofrequency ablation, and nine patients with transcatheter arterial chemoembolization.

Gd-EOB-MRI is the most sensitive procedure for the diagnosis of small HCCs [13, 14, 15, 16]. Kim et al. reported that the addition of Gd-EOB-MRI to dynamic CT was associated with the detection of additional HCC nodules in a significant proportion of patients, and resulted in a decreased risk of HCC recurrence and improved overall survival of patients who were initially assessed to have a single nodular HCC by dynamic CT [33]. Many studies have reported that the eradication of HCV reduces the risk of HCC [4, 5, 6, 34]. However, no studies have ruled out the presence of HCC by Gd-EOB-MRI before starting DAA therapy. If the only imaging modality used before treatment is multiphase dynamic CT or ultrasound, it is possible to miss a small lesion. All of our patients have been followed up with Gd-EOB-MRI, which should lead to more accurate results. In this study, the incidence rates of multistep hepatocarcinogenesis at 3 and 5 years were 11.2% and 21.2%, respectively. Viral eradication by DAA therapy suppressed multistep hepatocarcinogenesis, but the mechanism of this suppression is not clear.

In nine patients (13.3%), there was direct development to overt hypervascular HCC, known as de novo hepatocarcinogenesis. The cumulative incidence rates at 3 and 5 years were 2.0% and 3.6%, respectively. The rate of de novo hepatocarcinogenesis in this study was very low compared with a previous study by Yamamoto et al [35]. Their study excluded patients with hypervascular nodules, but did not exclude those with a history of HCC. Most patients with a history of HCC due to de novo hepatocarcinogenesis have intrahepatic metastases [36, 37]. High AFP-L3% values are associated with de novo hepatocarcinogenesis. It is well known that elevated AFP-L3% values are associated with tumor grade, including microsatellite lesions and HCC hypervascularity [38, 39]. Even if the total AFP value is low, careful surveillance may be required if the AFP-L3% value is high.

Our study has several limitations. First, some well-differentiated HCCs have been reported to show a high enhancement ratio on HBP [40]. If the signal intensity of a nodule does not decrease, there is a risk of mistaking multistep hepatocarcinogenesis for de novo hepatocarcinogenesis after imaging studies. However, the frequency of this error is considered to be low. Second, in this study only 33 resected nodules were examined pathologically. However, one of the major aims of the study was to define standard criteria for follow-up of NHHNs using imaging and not biopsy results.

In conclusion, hepatocarcinogenesis is often a multistep process, occurring via NHHNs, and patients with NHHNs should be carefully monitored using imaging modalities such as Gd-EOB-MRI. The eradication of HCV by DAA therapy reduces multistep carcinogenesis. Some patients instead exhibit de novo carcinogenesis, and are characterized by high AFP-L3% values.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010; 127: 2893-917.
2. Terrault NA, Hassanein TI. Management of the patient with SVR. J Hepatol. 2016; 65(1 Suppl): S120-S129.
3. El-Serag HB, Kanwal F, Richardson P, Kramer J. Risk of hepatocellular carcinoma after sustained virological response in Veterans with hepatitis C virus infection. Hepatology. 2016 Jul; 64(1):130-7.
4. Waziry R, Hajarizadeh B, Grebely J, Amin J, Law M, Danta M, George J, Dore GJ. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: A ystematic review, meta-analyses, and meta-regression. J Hepatol. 2017 Dec; 67:1204-1212.
5. Galati G, Muley M, Viganò M, Iavarone M, Vitale A, Dell'Unto C, Lai Q, Cabibbo G, Sacco R, Villa E, Trevisani F. Occurrence of hepatocellular carcinoma after direct-acting antiviral therapy for hepatitis C virus infection: literature review and risk analysis. Expert Opin Drug Saf. 2019; 18:603-610.
6. Piñero F, Mendizabal M, Ridruejo E, Herz Wolff F, Ameigeiras B, Anders M, Schinoni MI, Reggiardo V, Palazzo A, Videla M, Alonso C, Santos L, Varón A, Figueroa S, Vistarini C, Adrover R, Fernández N, Perez D, Tanno F, Hernández N, Sixto M, Borzi S, Bruno A, Cocozzella D, Soza A, Descalzi V, Estepo C, Zerega A, de Araujo A, Cheinquer H, Silva M; LALREAN. Treatment with direct-acting antivirals for HCV decreases but does not eliminate the risk of hepatocellular carcinoma. Liver Int. 2019; 39:1033-1043.
7. Choi JY, Lee JM, Sirlin CB. CT and MR imaging diagnosis and staging of hepatocellular carcinoma: part I. Development, growth, and spread: key pathologic and imaging aspects. Radiology. 2014; 272(3):635-54.
8. Van Beers BE, Pastor CM, Hussain HK: Primovist, Eovist: what to expect? J Hepatol 2012; 57:421–429.
9. Kudo M: Will Gd-EOB-MRI change the diagnostic algorithm in hepatocellular carcinoma? Oncology 2010; 78(Suppl 1):87–93.
10. Kudo M, Matsui O, Izumi N, Iijima H, Kadoya M, Imai Y, Liver Cancer Study Group of Japan: Surveillance and diagnostic algorithm for hepatocellular carcinoma proposed by the Liver Cancer Study Group of Japan: 2014 update. Oncology 2014; 87(Suppl. 1):7–21.
11. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu; European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol. 2018; 69(1):182-236.
12. Mitchell DG, Bruix J, Sherman M, Sirlin CB: LI-RADS (Liver Imaging Reporting and Data System): summary, discussion, and consensus of the LI-RADS Management Working Group and future directions. Hepatology 2015; 61:1056–1065.
13. Sun HY, Lee JM, Shin CI, Lee DH, Moon SK, Kim KW, et al. Gadoxetic acid-enhanced magnetic resonance imaging for differentiating small hepatocellular carcinomas (< or =2 cm in diameter) from arterial enhancing pseudolesions: special emphasis on hepatobiliary phase imaging. Invest Radiol 2010; 45:96–103.
14. Hanna RF, Miloushev VZ, Tang A, Finklestone LA, Brejt SZ, Sandhu RS, et al. Comparative 13-year meta-analysis of the sensitivity and positive predictive value of ultrasound, CT, and MRI for detecting hepatocellular carcinoma. Abdom Radiol 2016; 41:71–90.
15. Inoue T, Kudo M, Komuta M, Hayaishi S, Ueda T, Takita M, et al. Assessment of Gd-EOB-DTPA-enhanced MRI for HCC and dysplastic nodules and comparison of detection sensitivity vs. MDCT. J Gastroenterol 2012; 47:1036–1047.
16. Duncan JK, Ma N, Vreugdenburg TD, Cameron AL, Maddern G. Gadoxetic acid-enhanced MRI for the characterization of hepatocellular carcinoma: A systematic review and meta-analysis. J Magn Reson Imaging 2017; 45:281–290.
17. Kumada T, Toyoda H, Tada T, Sone Y, Fujimori M, Ogawa S, Ishikawa T. Evolution of hypointense hepatocellular nodules observed only in the hepatobiliary phase of gadoxetate disodium-enhanced MRI. AJR Am J Roentgenol. 2011 Jul; 197(1):58-63.
18. Hyodo T, Murakami T, Imai Y, Okada M, Hori M, Kagawa Y, Kogita S, Kumano S, Kudo M, Mochizuki T. Hypovascular nodules in patients with chronic liver disease: risk factors for development of hypervascular hepatocellular carcinoma. Radiology. 2013 Feb; 266(2):480-90.
19. Kokudo N, Takemura N, Hasegawa K, Takayama T, Kubo S, Shimada M, Nagano H,Hatano E, Izumi N, Kaneko S, Kudo M, Iijima H, Genda T, Tateishi R, Torimura T, Igaki H, Kobayashi S, Sakurai H, Murakami T, Watadani T, Matsuyama Y. Clinical practice guidelines for hepatocellular carcinoma: The Japan Society of Hepatology 2017 (4th JSH-HCC guidelines) 2019 update. Hepatol Res. in press
20. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M; APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 200; 43(6):1317-25.
21. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and fibrotest. Hepatology. 2007 Jul; 46(1):32-6.
22. Johnson PJ, Berhane S, Kagebayashi C, Satomura S, Teng M, Reeves HL, O'Beirne J, Fox R, Skowronska A, Palmer D, Yeo W, Mo F, Lai P, Iñarrairaegui M, Chan SL, Sangro B, Miksad R, Tada T, Kumada T, Toyoda H. Assessment of liver function in patients with hepatocellular carcinoma: a new evidence-based approach-the ALBI grade. J Clin Oncol. 2015 20; 33(6):550-8.
23. Kagebayashi C, Yamaguchi I, Akinaga A, Kitano H, Yokoyama K, Satomura M, Kurosawa T, Watanabe M, Kawabata T, Chang W, Li C, Bousse L, Wada HG, Satomura S.Automated immunoassay system for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis. Anal Biochem. 2009 15; 388:306-11.
24. Motosugi U, Murakami T, Lee JM, Fowler KJ, Heiken JP, Sirlin CB; LI-RADS HBA Working Group. Recommendation for terminology: Nodules without arterial phase hyperenhancement and with hepatobiliary phase hypointensity in chronic liver disease. J Magn Reson Imaging. 2018; 48:1169-1171.
25. Sakamoto M, Effendi K, Masugi Y. Molecular diagnosis of multistage hepatocarcinogenesis. Jpn J Clin Oncol. 2010; 40(9):891-6.
26. Kudo M. Multistep human hepatocarcinogenesis: correlation of imaging with pathology. J Gastroenterol. 2009; 44 Suppl 19:112-8.
27. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999; 94:496- 509.
28. Kanda Y. Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. Bone Marrow Transplant. 2013; 48: 452-458.
29. Narita M, Hatano E, Arizono S, Miyagawa-Hayashino A, Isoda H, Kitamura K, Taura K, Yasuchika K, Nitta T, Ikai I, Uemoto S. Expression of OATP1B3 determines uptake of Gd-EOB-DTPA in hepatocellular carcinoma. J Gastroenterol. 2009; 44:793-8.
30. Kitao A, Zen Y, Matsui O, Gabata T, Kobayashi S, Koda W, Kozaka K, Yoneda N, Yamashita T, Kaneko S, Nakanuma Y. Hepatocellular carcinoma: signal intensity at gadoxetic acid-enhanced MR Imaging--correlation with molecular transporters and histopathologic features. Radiology. 2010; 256(3):817-26.
31. Kitao A, Matsui O, Yoneda N, Kozaka K, Shinmura R, Koda W, Kobayashi S, Gabata T, Zen Y, Yamashita T, Kaneko S, Nakanuma Y. The uptake transporter OATP8 expression decreases during multistep hepatocarcinogenesis: correlation with gadoxetic acid enhanced MR imaging. Eur Radiol. 2011; 21(10):2056-66.
32. Midorikawa Y, Takayama T, Shimada K, Nakayama H, Higaki T, Moriguchi M, Nara S, Tsuji S, Tanaka M. Marginal survival benefit in the treatment of early hepatocellular carcinoma. J Hepatol. 2013; 58(2):306-11.
33. Kim HD, Lim YS, Han S, An J, Kim GA, Kim SY, Lee SJ, Won HJ, Byun JH. Evaluation of early-stage hepatocellular carcinoma by magnetic resonance imaging with gadoxetic acid detects additional lesions and increases overall survival. Gastroenterology. 2015; 148(7):1371-82.
34. Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. J Hepatol. 2017 Sep 5. pii: S0168-8278(17)32273-0.
35. Yamamoto A, Ito K, Tamada T, Higaki A, Kanki A, Sato T, Tanimoto D. Newly developed hypervascular hepatocellular carcinoma during follow-up periods in patients with chronic liver disease: observation in serial gadoxetic acid-enhanced MRI. AJR Am J Roentgenol. 2013; 200(6):1254-60.
36. Toyoda H, Kumada T, Tada T, Niinomi T, Ito T, Sone Y, Kaneoka Y, Maeda A. Non-hypervascular hypointense nodules detected by Gd-EOB-DTPA-enhanced MRI are a risk factor for recurrence of HCC after hepatectomy. J Hepatol. 2013; 58(6):1174-80.
37. Toyoda H, Kumada T, Tada T, Mizuno K, Sone Y, Kaneoka Y, Maeda A, Akita T, Tanaka J. Impact of previously cured hepatocellular carcinoma (HCC) on new development of HCC after eradication of hepatitis C infection with non-interferon-based treatments. Aliment Pharmacol Ther. 2018; 48(6):664-670.
38. Kumada T, Nakano S, Takeda I, Kiriyama S, Sone Y, Hayashi K, Katoh H, Endoh T, Sassa T, Satomura S: Clinical utility of *Lens culinaris* agglutinin-reactive alpha-fetoprotein in small hepatocellular carcinoma: special reference to imaging diagnosis. J Hepatol 1999; 30:125–130.
39. Tada T, Kumada T, Toyoda H, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, Kitabatake S, Kuzuya T, Nonogaki K, Shimizu J, Yamaguchi A, Isogai M, Kaneoka Y, Washizu J, Satomura S: Relationship between *Lens* *culinaris* agglutinin-reactive α-fetoprotein and pathologic features of hepatocellular carcinoma. Liver Int 2005; 25:848–853.
40. Asayama Y, Tajima T, Nishie A, Ishigami K, Kakihara D, Nakayama T, Okamoto D, Fujita N, Aishima S, Shirabe K, Honda H. Uptake of Gd-EOB-DTPA by hepatocellular carcinoma: radiologic-pathologic correlation with special reference to bile production. Eur J Radiol. 2011; 80(3):e243-8.

Figure Legends

Figure 1, Flowchart of the patient selection process.

Gd-EOB-MRI: gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid–enhanced magnetic resonance imaging, HCV: hepatitis C virus, HCC: hepatocellular carcinoma, DAA: direct-acting antiviral.

Figure 2, Multistep hepatocarcinogenesis observed by Gd-EOB-MRI (60s, male).

A nonhypervascular hypointense nodule (NHHN) appeared during the hepatobiliary phase in September 2015 (arrowhead). The hypervascular foci in the NHHN appeared during the arterial phase in October 2015 (arrow). Gd-EOB-MRI: gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid–enhanced magnetic resonance imaging, DAA: direct-acting antiviral.

Figure 3, De novo hepatocarcinogenesis observed by Gd-EOB-MRI (70s, female).

A hypervascular nodule appeared during the arterial phase without preexisting No nonhypervascular hypointense nodule (NHHN) in September 2015 (arrow). Gd-EOB-MRI: gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid–enhanced magnetic resonance imaging, DAA: direct-acting antiviral.

Figure 5, The frequencies of multistep and de novo hepatocarcinogenesis.

Multistep hepatocarcinogenesis occurred in 58 patients (86.6%), while de novo hepatocarcinogenesis occurred in nine patients (13.4%). Among the latter, seven patients showed hypervascular nodules in the liver without preexisting NHHNs, and the remaining two patients demonstrated hypervascular nodules at a different site from the NHHNs.

Figure 4, Cumulative incidence of multistep and de novo hepatocarcinogenesis.

The incidence rates of multistep and de novo hepatocarcinogenesis were 11.2% and 2.0% at 3 years, respectively, and 21.2% and 3.6% at 5 years, respectively.

Figure 6, Cumulative incidence of multistep hepatocarcinogenesis in patients with or without DAA therapy.

The incidence rates with and without DAA therapy were 6.9% and 14.9% at 3 years, respectively, and 9.0% and 26.4% at 5 years, respectively. DAA: direct-acting antiviral.

Figure 7, Cumulative incidence of de novo hepatocarcinogenesis according to AFP-L3% values.

The cumulative incidence rates of AFP-L3% ≥ 5% and < 5% were 7.9% and 0.3% at 3 years, respectively, and 13.8% and 0.3% at 5 years, respectively. AFP-L3: *Lens culinaris* agglutinin–reactive fraction of alpha-fetoprotein.