Received: 13 June 2020 | Revised: 24 August 2020 | Accepted: 25 August 2020

DOI: 10.1002/ccr3.3360

CASE REPORT

Clinical Case Reports Open Access WILEY

# Hepatitis B virus core–related antigen is useful for surveillance of hepatocellular carcinoma recurrence in a patient with occult hepatitis B virus infection: Case report

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**Abstract**

Serum HBV core–related antigen (HBcrAg) is useful for detecting HCC in patients with occult HBV infection. Surveillance for HCC is needed in patients who are positive for HBcrAg, even if they are negative for HBsAg and HBV DNA.

**KEYWORDS**

hepatitis B core-related antigen, hepatocellular carcinoma, occult HBV infection

## 1 | INTRODUCTION

A 73-year-old woman with occult hepatitis B virus (HBV) infection was diagnosed with hepatocellular carcinoma (HCC). Serum HBV core–related antigen (HBcrAg) was elevated, and HBV covalently closed circular DNA (cccDNA) was present in the liver tissue. This report shows HBcrAg might be a predictive factor for the development of HCC.

It is estimated that 2 billion people worldwide have hepatitis B virus (HBV) infection, 250 million people have persistent HBV infection, and 0.7 million people die of HBV infection–related diseases annually.[1] HBV is a risk factor for the development of hepatocellular carcinoma (HCC) and is one of the very important diseases from the viewpoint of not only treatment when HCC occurs but also prevention of carcinogenesis and recurrence.[2]

Patients with a positive test result for hepatitis B surface antigen (HBsAg) are often treated with a nucleic acid analog (NA) preparation for the suppression of progressing liver fibrosis and hepatocarcinogenesis. However, HBV DNA may be detected in serum or liver tissue in spite of a negative test result for serum HBsAg. In addition, HBsAg gene mutations can occur resulting in HBsAg escape mutants that are not recognized by HBs antibodies.[3] These patients are considered as having occult HBV infection (OBI). OBI can also be a risk factor for hepatocarcinogenesis; therefore, it is important to detect it at an early stage. However, some patients with OBI have HBV DNA detectable in the blood, whereas others have HBV detectable only in the liver tissue. It may be difficult to detect OBI by means of normal surveillance.[4] Patients with OBI are often found to have advanced HCC because they have not undergone HCC surveillance. We report a case of a patient with OBI in whom serum HBV core–related antigen (HBcrAg) was useful for the surveillance of HCC recurrence.

## 2 | CASE HISTORY/ EXAMINATION

A 73-year-old woman presented to our facility for follow-up. She had varicose veins in the right leg and no history of alcohol consumption or smoking. She had been found to be HBsAg-positive in the late 1980s and was attending a clinic for following the liver function. The HBV infection status of her mother was unknown. In June 2007 in her previous hospital, she was found to have a single HCC with a diameter of 15 mm at segment 4 of the liver, and radiofrequency ablation (RFA) was performed. Laboratory investigation results at that time were as follows: HBsAg-positive, hepatitis B envelope antigen (HBeAg)–negative, hepatitis B envelope antibody (HBeAb)–positive, and HBV DNA titer of 1840 IU/mL. At the patient's insistence, she did not receive antiviral treatment and was carefully followed up by her previous doctor. Two years later, her HBV DNA titer dropped below 1300 IU/mL. Another 2 years thereafter, her HBsAg became negative, and her HBV DNA became undetectable. She had no recurrence of HCC during this 4-year period; however, about 7 years after the initial treatment, a recurrence of HCC with a diameter of 18 mm was seen at segment 8 of the liver. As a result, she was referred to and hospitalized at our department for the treatment of HCC. We ruled out the possibility that the recurrence was spread from the prior segment 4 lesion because there had been no recurrence for 7 years and the new lesion was not in contact with the previous treatment site (Figure 1C). We proposed tumor resection to the patient since the tumor was a single recurrence of 2 cm or less. However, the patient did not want the surgical treatment due to the possibility of whose activities of daily living (ADL) has weakened after treatment. Therefore, we chose to perform transcatheter arterial chemoembolization (TACE) and RFA at the time.

The patient had no significant symptoms on admission. Laboratory data on admission showed no abnormalities in blood count, coagulation system, general biochemical tests, and liver fibrosis markers. As for tumor markers, the patient's total α-fetoprotein and des-γ-carboxy prothrombin were normal, but her α-fetoprotein-L3 fraction was as high as 43.9% (Table 1). With regard to virus markers, she was HBsAg-negative, HBeAg-negative, HbeAb-positive, and HBcAb-positive. Furthermore, her HBV DNA was not detectable at <1300 IU/mL, and her HBV core–related antigen (HBcrAg) was elevated at 3.7 log U/mL (normal value, < 3.0 log U/mL) (Table 2). Abdominal contrast-enhanced computed tomography revealed a high-low pattern tumor lesion having a diameter of 18 mm at segment 8 of the liver (Figure 1A). In addition, abdominal contrast-enhanced magnetic resonance imaging (MRI) revealed that the patient's hepatic morphology was almost normal, and a high-low pattern was observed in dynamic imaging with

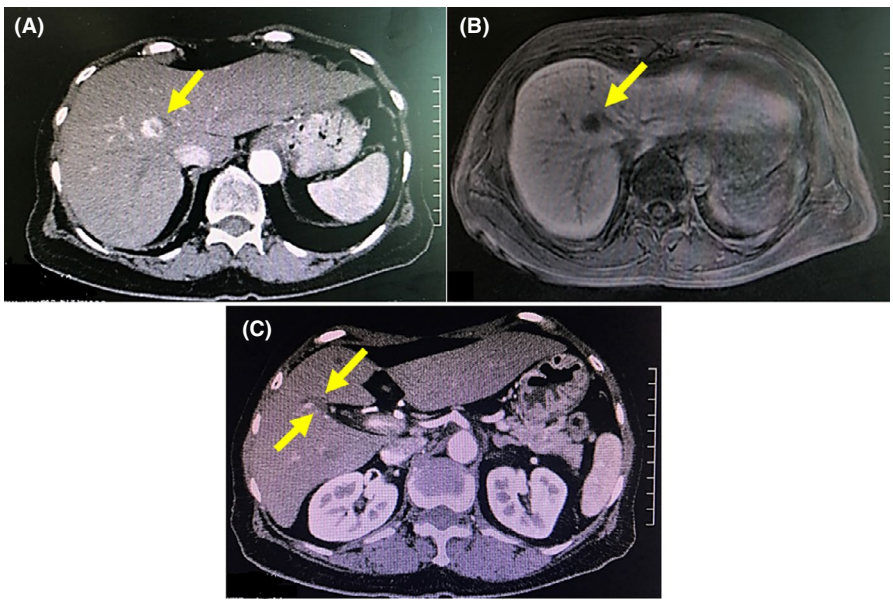


**FIGURE 1** A, Abdominal contrast-enhanced computed tomographic scan in early phase. Liver morphology is almost normal with no splenomegaly. A high-low pattern lesion with a diameter of 18 mm was observed in segment 8 of the liver (*arrow*); therefore, hepatocellular carcinoma (HCC) recurrence was diagnosed. B, Abdominal contrast-enhanced magnetic resonance imaging scan in hepatobiliary phase. The hepatic morphology was almost normal. A high-low pattern was observed in dynamic imaging with a diameter of 18 mm in segment 8 of the liver, and a neoplastic lesion that was a contrast-deficient image in the hepatocyte contrast phase was recognized (*arrow*), confirming HCC recurrence. C, Abdominal contrast-enhanced computed tomographic scan in early phase. The RFA treatment scar of segment 4 performed at the previous hospital (*arrow*) was not in contact with the new lesion of segment 8

**TABLE 1** Laboratory data on admission

| **Hematology** | | **Blood chemistry** | |
|---|---|---|---|
| WBC | 4700/μL | TP | 6.7 g/dL |
| RBC | 406 × $10^4$/μL | Alb | 4.0 g/dL |
| Hemoglobin | 12.9 | T-bil | 0.7 mg/dL |
| Hematocrit | 96.3% | AST | 16 U/L |
| Platelet | 16.8 × $10^4$/μL | ALT | 10 U/L |
| **Coagulation** | | LDH | 188 U/L |
| PT | 83% | ALP | 160 U/L |
| INR | 1.09 | GGT | 14 U/L |
| APTT | 25.5 s | ChE | 306 U/L |
| Fibrinogen | 344 mg/dL | BUN | 15 mg/dL |
| **Hepatic fibrosis markers** | | Cre | 0.57 mg/dL |
| Hyaluronic acid | 35.6 ng/mL | HbA1c | 5.9% |
| Type IV collagen 7S | 4.7 ng/mL | **Tumor markers** | |
| | | AFP | 3.0 ng/mL |
| | | AFP-L3 affinity | 43.9% |
| | | DCP | 20 mAU/mL |

Abbreviations: AFP, α-fetoprotein; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine transaminase; APTT, activated partial thromboplastin time; AST, aspartate transaminase; BUN, blood urea nitrogen; ChE, cholinesterase; Cre, creatinine; DCP, des-γ-carboxy prothrombin; GGT, γ-glutamyl transpeptidase; INR, international normalized ratio; L3, lectin 3; LDH, lactate dehydrogenase; PT, prothrombin time; RBC, red blood cell count; T-bil, total bilirubin; TP, total protein; WBC, white blood cell count.

a diameter of 18 mm in segment 8 of the liver. It was diagnosed as a neoplastic lesion because of its contrast-deficient appearance in the hepatocyte contrast phase. Therefore, HCC recurrence was diagnosed (Figure 1B). Hepatic arteriography revealed a tumor stain from the anterior superior branch of the right hepatic artery (A8) (Figure 2A). TACE was performed from A8 using 50 mg of a cisplatin formulation for intravenous infusion (IA Coal®). After TACE, RFA was performed in a spherical shape with a diameter of 3 cm

**TABLE 2** Viral markers on admission

| **Viral marker** | **Value** |
|---|---|
| HBsAg | – |
| HbeAg | – |
| HbeAb | + |
| HBcAb | + |
| HBsAb | – |
| HBV DNA | < 1300 copies/mL |
| HBcrAg | 3.7 log U/mL |
| HCVAb | – |

Abbreviations: Ab, antibody; Ag, antigen; HBc, hepatitis B core; HBcrAg, HBV core–related antigen; HBe, hepatitis B envelope; HBs, hepatitis B surface; HBV, hepatitis B virus; HCV, hepatitis C virus.

to surround the TACE area (Figure 2B, C). Abdominal contrast computed tomography after treatment confirmed that Lipiodol contrast agent (Guerbet) was well accumulated at the tumor site and that a sufficient ablation margin was secured (Figure 3).

## 2.1 | Outcome and follow-up

The patient was discharged after completing HCC treatment. She has been followed up for 5 years and has not shown any signs of recurrence. Her HBsAg has remained negative, and HBV DNA has not been detected; however, her HBcrAg has been slightly elevated. Currently, she is being monitored without any medication.

# 3 | DISCUSSION

Transcatheter arterial chemoembolization ensures a high intratumor concentration of chemotherapy agent and subsequently intratumor hypoxia and necrosis. Moreover, Lipiodol is combined with the chemotherapeutic agent for its well-proved embolic effect, but it was shown that it can prolong

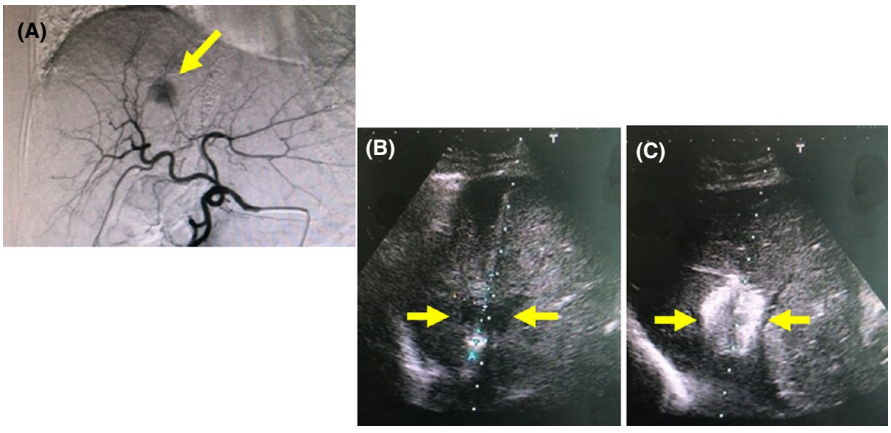


**FIGURE 2** A, Abdominal angiographic findings. In hepatic arteriography, a tumor stain was observed from the anterior superior branch of right hepatic artery 8 (*arrow*). B, C, Radiofrequency ablation was performed in a spherical shape with a diameter of 3 cm to surround the transarterial chemoembolization area (*arrows*)

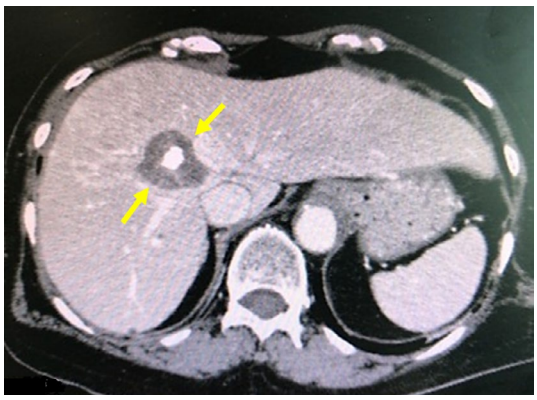

**FIGURE 3** Abdominal contrast-enhanced computed tomography after treatment confirmed that Lipiodol was well accumulated at the tumor embolus and a sufficient ablation margin was secured (*arrows*)

the intratumor passage of the drug. The rate of curative resectability significantly increases after TACE treatment, independent of the chemotherapeutic drugs used.[5] However, this time, the patient refused hepatectomy, so we added RFA and aimed for radical treatment.

In 2008, the European Association for the Study of the Liver defined OBI as the "presence of HBV DNA in the liver (with detectable or undetectable HBV DNA in the serum) of individuals testing HBsAg negative by currently available assays."

In this case, although the patient's serum HBsAg and HBV DNA became negative after an initial treatment for HCC, she had tumor recurrence 7 years after treatment. The patient's clinical course from the initial occurrence of HCC is shown in Figure 4. Her HBV DNA titer decreased to <1300 IU/mL from 2 years after the initial treatment; HBsAg became negative 4 years after the initial treatment; and HBV DNA became undetectable. Therefore, this case was assumed to be one of HCC recurrence after OBI. Upon admission, the patient's HBsAg was negative, and her HBV DNA was not detected. However, her HBcrAg was as high as 3.7 log U/mL. HBcrAg is known to correlate with the amount of covalently closed circular DNA (cccDNA) in hepatocytes, and it has been reported that HBcrAg may be useful as a criterion for stopping NA preparations.[6,7] In addition, its usefulness in predicting HCC occurrence and recurrence with the use of NA has been reported.[8] In this case, on the basis of nontumor liver biopsy performed during RFA, quantitative measurement of HBV cccDNA revealed 1.8 log copies/μg in liver tissue. It is known that HBV DNA is present in liver tissue at a high rate even if serum HBV DNA is negative.[9] Therefore, our patient's case was confirmed to be OBI.

The association of OBI with hepatocarcinogenesis has been reported.[4,10-12] In an analysis of 53 HBsAg-negative patients with unexplained HCC, Wong et al reported that cccDNA was detected in liver tissue of 29 (47%) patients.[13] On the basis of these reports, it was presumed that our patient also likely developed carcinogenesis from OBI. The scheme for HBV infection and the pathway of hepatocarcinogenesis are shown in Figure 5. Two major pathways are considered for hepatocellular carcinogenesis. The first is through accumulation of hepatocellular DNA damage due to oxidative stress during the progression of chronic inflammation and fibrosis.[14-17] The second is through a direct carcinogenesis mechanism by HBV; that is, HBx protein and HBV DNA activate transcription factors such as nuclear factor-κB and promote the production of cytokines such as tumor necrosis factor-α and interleukin-6 by incorporation into the cell genome, thereby affecting cell proliferation and apoptosis.[18-21] Our patient had few chronic inflammatory changes in liver morphology, and her hepatobiliary enzymes and liver fibrosis markers were normal. Therefore, it is considered that the direct carcinogenic mechanism of HBV is more likely to be the trigger for her hepatocarcinogenesis. There are several reports on the inhibitory effect of NA on hepatocarcinogenesis in chronic hepatitis B.[2,22] However, there is no clear evidence

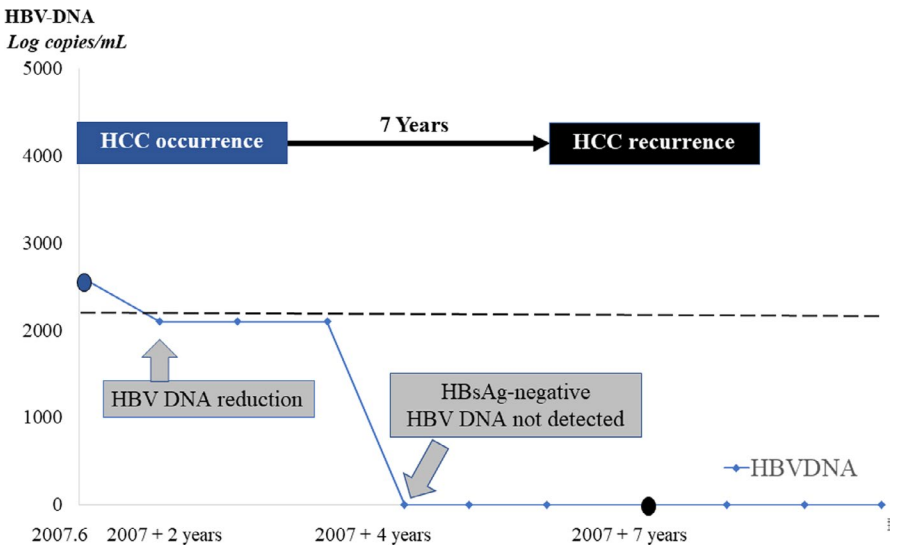


**FIGURE 4** The clinical course of hepatocellular carcinoma (HCC) in this case. In June 2007, the patient was found to have HCC occurrence, and radiofrequency ablation (RFA) was performed. Two years later, the patient's hepatitis B virus (HBV) DNA titer dropped below 2100 copies/mL. Another 2 y later, her hepatitis B surface antigen (HBsAg) became negative, and her HBV DNA became undetectable. However, about 7 y after the initial treatment, a recurrence of HCC was seen in the liver

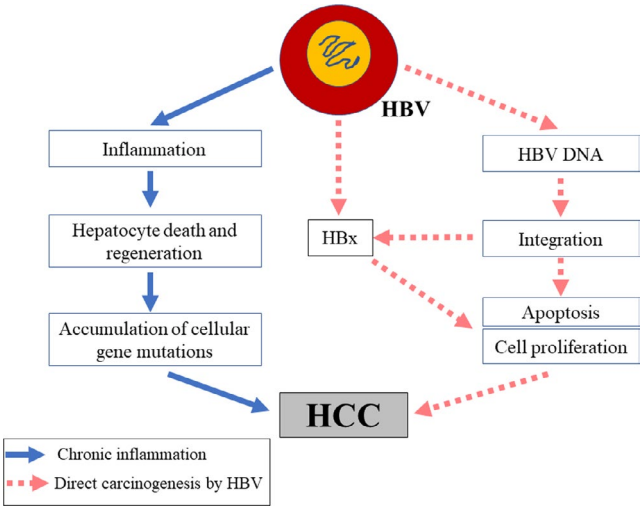


**FIGURE 5** The scheme for hepatitis B virus (HBV) infection and the pathway of hepatocellular carcinoma (HCC) carcinogenesis

of whether hepatic carcinogenesis can be suppressed by administering NA in patients with only a high HBcrAg. Our patient was seen in follow-up without any medication (with her consent), and she has been recurrence-free for about 5 years. However, it is necessary to continue careful follow-up, considering her history of HCC recurrence 7 years after her first case. Currently, several drugs that eliminate HBV cccDNA in hepatocytes are being developed,[23-26] and it is strongly hoped that such drugs will soon be approved for clinical use in the near future.

To conclude, HBcrAg is useful for detecting HCC recurrence in patients with OBI. Surveillance for HCC is needed in patients who are positive for serum HBcrAg, even if they are negative for HBsAg and HBV DNA.

## ACKNOWLEDGMENTS

We thank Editage (www.editage.com) for English language editing. We also express our gratitude to Waki Nagashima, Yuki Nozaki, Atsuko Ishibashi, Nao Kodama, Rina Akahoshi, Chihiro Tanaka, Akiko Tanaka, Motoko Kawashima, and Tomoko Nagaura for their invaluable support.

## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

## AUTHOR CONTRIBUTIONS

All authors participated in data collection. KY contributed to the manuscript. All authors read and approved the final manuscript.

## ETHICAL APPROVAL

This patient has given written informed consent to publish the case, including publication of images.

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**How to cite this article:** Yokoyama K, Yamauchi E, Uchida Y, et al. Hepatitis B virus core–related antigen is useful for surveillance of hepatocellular carcinoma recurrence in a patient with occult hepatitis B virus infection: Case report. *Clin Case Rep*. 2020;8:3031–3036. https://doi.org/10.1002/ccr3.3360