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【 CASE REPORT 】

Diagnosis and Resection Treatment of Triplet Hepatocellular Carcinomas with a non-B non-C Background in a Middle Aged Man over a Period of 6-years

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Abstract:
We report a 71-year-old man with non-B non-C chronic liver damage who had been regularly visiting our hospital since he was 38 years of age. He underwent three partial hepatectomies for hepatocellular carcinoma (HCC) diagnosed at 65, 67, and 71 years of age, respectively. A histopathological examination showed moderately-differentiated HCC, and chronic hepatitis with mild fibrosis stage in non-tumor areas. alpha-fetoprotein (AFP) and PIVKAII were not useful for the early prediction of HCC, but *TERT* promotor mutation (C228T) in serum cell-free DNA was useful. This is the first report on the importance of long-term follow-up in non-B non-C chronic liver damage, regardless of the fibrosis stage.

Key words: hepatocellular carcinoma, non-B non-C, mild fibrosis stage, *TERT* promoter, cell-free DNA, Wild-type blocking PCR

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Introduction

The main causes of hepatocellular carcinoma (HCC) are hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in both Japan and most other countries (1, 2). The development of HCC correlates with the progression of liver fibrosis, and the rate of future carcinogenesis is predicted by the degree of fibrosis (3, 4). HCC is rare in subjects with normal or mild fibrosis of the liver (5-8). We herein report triplet HCCs, diagnosed at ages 65, 67 and 71 years in a patient with non-B non-C liver injury and mild fibrosis. Each tumor was excised surgically soon after diagnosis and thereafter was examined histopathologically.

Case Report

The patient was a 71-year-old man who had been regularly visiting our hospital for about 30 years due to chronic liver disease. On the first consultation at 38 years of age, he had no other remarkable medical history. HBV surface antigen was negative, but HBV surface antibody and HBV core antibody were positive. HCV antibody was negative. Antinuclear antibody and anti-mitochondrial antibody were negative, and alcohol consumption was less than 20 g/day (namely, non-B non-C liver damage). The established diagnosis was non-alcoholic fatty liver disease diagnosed by ultrasonography and a past history of HBV infection. Treatment included advice to refrain from alcohol drinking and diet/exercise therapy. Subsequent follow-up showed an improvement of the liver damage based on the results of blood tests, including an improvement of fatty liver as confirmed by abdominal ultrasonography (US). The patient was followed-up annually by abdominal US and/or dynamic computed tomography (CT).

Liver function tests showed a progressive decrease in the

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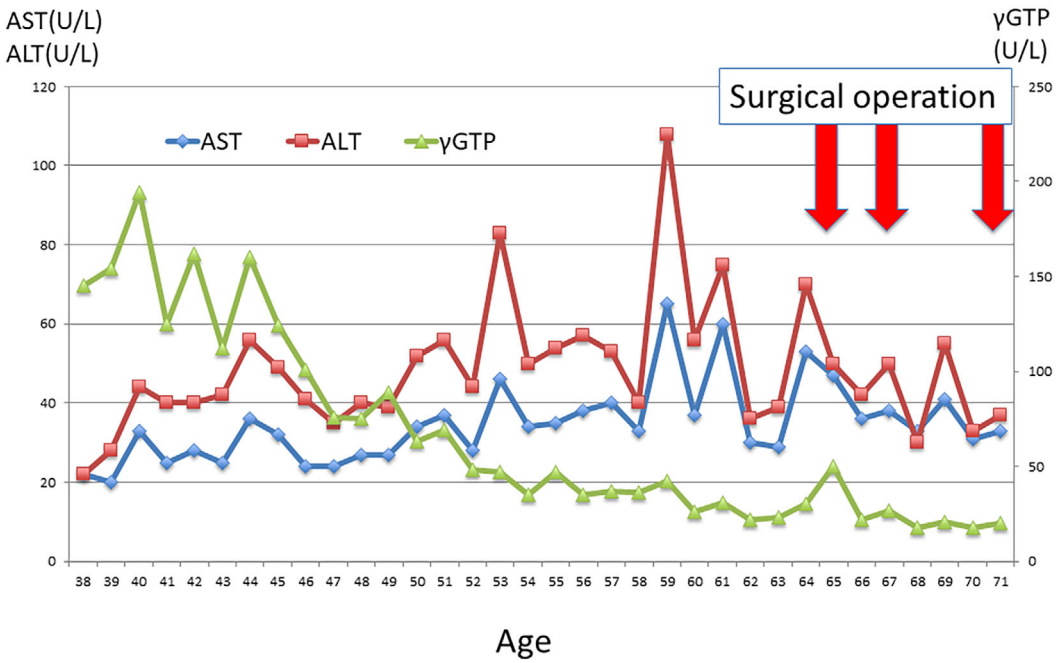

Figure 1. Changes in the liver function tests since the first consultation. The serum γGTP levels decreased gradually, but returned to the normal range following the excision of HCCs. On the other hand, the levels of AST and ALT were within the mildly abnormal levels. The patient underwent partial hepatectomy at ages 65, 67, and 71.

γ-glutamyl transpeptidase (γGTP) levels, but they subsequently improved to normal levels, while aspartate aminotransferase (AST) and alanine aminotransferase (ALT) remained persistently close to the upper normal or slightly abnormal (Fig. 1). There was no significant change in body weight since first consultation, and blood tests did not show any increase in either alpha-fetoprotein (AFP) or protein induced by vitamin K absence of antagonist (PIVKA) II (Table).

At 65 years of age, a follow-up abdominal US showed a solitary liver tumor at S5. A confirmatory dynamic CT identified the tumor in S5 measuring 20×18-mm in size, with a contrast effect in the arterial phase and washout in the portal vein phase. EOB-MRI showed a highly heterogeneous mass on T2-weighted imaging (T2WI) and diffusion-weighted imaging (DWI), and no uptake in the hepatobiliary phase. Based on these features, the tumor was diagnosed as HCC. The patient underwent S5 partial hepatectomy and a histopathological examination of the resected tumor showed moderately organized HCC (negative surgical margin and no vascular invasion), with features of chronic hepatitis (CH) [fibrosis 1 / activity 1 (F1/A1)] in the non-tumorous areas.

At 67 years of age, a new liver tumor was detected at S7 on annual screening abdominal US. Dynamic CT showed a 20×20×15-mm tumor in the S7 segment of the liver, with a contrast effect in the arterial phase and washout in the portal vein phase. EOB-MRI showed a highly heterogeneous mass on T2WI and DWI, and no uptake in the hepatobiliary phase. The new tumor was accordingly diagnosed to be HCC and was consequently excised through S7 partial hepatectomy. Histopathologically, the resected tumor showed moderately organized HCC (negative surgical margin and no vascular invasion), surrounded by CH (F1/A1) non-tumor tissue.

At 71 years of age, another solitary HCC was diagnosed in the liver at segment S8 measuring 18 mm×18 mm×14 mm in size on dynamic CT and EOB-MRI. The contrast pattern was not different from the previous one. He underwent S8 partial hepatectomy and a histopathological examination of the excised specimen showed moderately organized HCC (negative surgical margin and no vascular invasion) with CH (F1/A1) in the non-tumor surrounding tissue. The postoperative clinical course was uneventful and the patient is still alive with no signs of recurrence several months after the third surgery.

Before the three partial hepatectomies, no differences were noted in the contrast pattern between the CT and MRI. All tumor specimens were moderately differentiated HCC e.g., fc(+), s0, vp0, vv0, va0, b0, im0, p0, sm(-) (Fig. 2). Furthermore, the non-tumor tissue in the specimen showed < 3% steatosis and mild fibrosis (F1) (Fig. 3).

Recently, we developed a highly sensitive method for the detection of *TERT* promotor mutations using wild-type blocking polymerase chain reaction (PCR) (WTB-PCR), combined with Sanger sequencing, and demonstrated its clinical usefulness for the early prediction of HCC by measuring TERT C228T in serum cell-free DNA (cfDNA). The sequencing analysis of WTB-PCR product demonstrated a detection limit in excess of 0.7% Mutant-type DNA in the background of Wild-type DNA (9). In the present case, the relationship between HCC and TERT C228T in serum cfDNA was serially examined by WTB-PCR. TERT C228T

Table. Summary of the Clinicopathological Data.

	First consultation	First partial hepatectomy	Second partial hepatectomy	Third partial hepatectomy
Age (years)	38	65	67	71
Wt (kg)/Ht (m)/BMI (kg/m^2)	57/1.6/22.4	57.5/1.6/22.8	57/1.6/22.5	56/1.6/22.2
AST/ALT (U/L)	22/22	46/62	37/52	36/40
γGTP (U/L)	145	23	50	20
Albumin (g/dL)	4.5	4.0	3.8	4.5
Total bilirubin (mg/dL)	0.5	0.7	0.5	0.8
Platelet (×10^4/μL)	34.3	21.1	21.8	19.5
Prothrombin time (%)	ND	84.8	99.3	96.5
Total cholesterol (mg/dL)	178	155	136	156
Triglyceride (mg/dL)	143	203	91	131
HbA1c (%)	6.5	6.0	6.1	6.0
Alpha-fetoprotein (μg/L)	5	5	4	4
PIVKAII (AU/L)	ND	14	12	15
HBsAg	negative	negative	negative	negative
HBsAb	positive	positive	positive	positive
HBcAb	positive	positive	positive	positive
HBV DNA (PCR)	negative	negative	negative	negative
HCV Ab	negative	negative	negative	negative
Fib-4 index	0.52	1.81	1.64	2.02
Liver histopathology				
Tumor tissue				
Occupation site		S5	S7	S8
Size (mm)		20×18	20×15×15	18×18×14
Gross findings		simple nodule	simple nodule	simple nodule
Organizational type		moderately	moderately	moderately
Organizational structure		trabecular	trabecular	trabecular
Development style		expansive	expansive	expansive
Capsule formation		+	+	+
Cancerous infiltration of the capsule		+	-	-
Formation of fibrous septum within the tumor		+	+	-
Invasion of the serosa		-	-	-
Vascular invasion		vp0/vv0/va0	vp0/vv0/va0	vp0/vv0/va0
Bile duct invasion		0	0	0
Intrahepatic metastasis		0	0	0
Surgical margins		sm- 5mm	sm- 9mm	sm- 5mm
Non-tumor tissue		CH	CH	CH
Steatosis (Grade /%)		0 /<3%	0 /<1%	0 /<1%
Fibrosis stage		1	1	1
Activity		1	1	1

Ht: height, Wt: weight, BMI: body mass index, ND: not determined, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HBcAb: hepatitis B core antibody, HBsAb: hepatitis B surface antibody, HBsAg: hepatitis B surface antigen, HCV: hepatitis C virus

was negative before the diagnosis of HCC 64 years of age, but it became positive at the time that HCC was diagnosed, namely when the patient was 65 years of age (Fig. 4).

Discussion

To the best of our knowledge, this is the first report on the importance of a long-term follow-up in non-B non-C chronic liver damage, regardless of the fibrosis stage. Furthermore, this report described three separate onsets of HCC which were diagnosed, excised surgically, and examined histopathologically in a single middle-aged patient within the span of 6 years. The histopathological findings of all three tumors were moderately differentiated HCCs with non-B non-C chronic liver damage and mild fibrosis. While several studies of HCC with a background of non-B non-C chronic liver damage and normal or mild fibrotic liver have been reported (5-8), triple hepatocarcinogenesis is rare. In our patient, all three of the tumors were solitary, measuring ≤20 mm in diameter, with a relatively long inter-new-tumor interval, and all showed moderate differentiation with well-differentiated features in some. Based on these features, we consider all three of these to be new tumors, arising at the age of 65, 67 and 71, rather than for the latter two to be in-

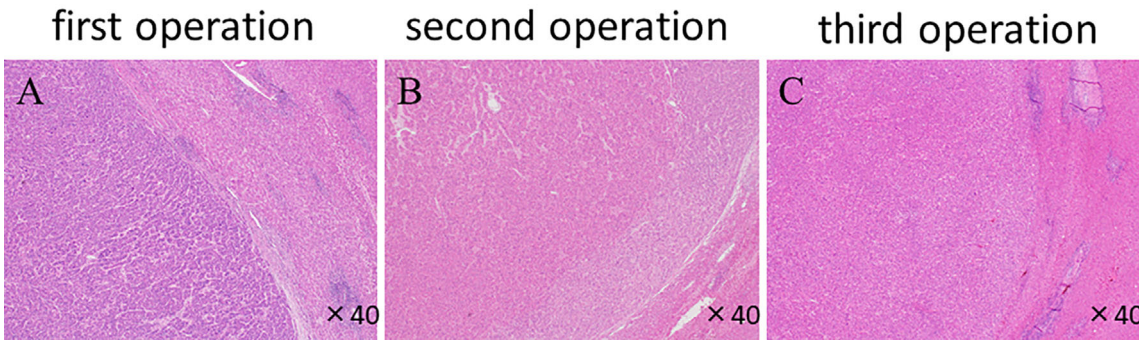

Figure 2. **Histopathological findings of the tumor areas of the resected specimens (Hematoxylin and Eosin staining ×40). All tumors showed features of moderately differentiated HCC [e.g., fc (+), s0, vp0, vv0, va0, b0, im0, p0, sm (-)].**

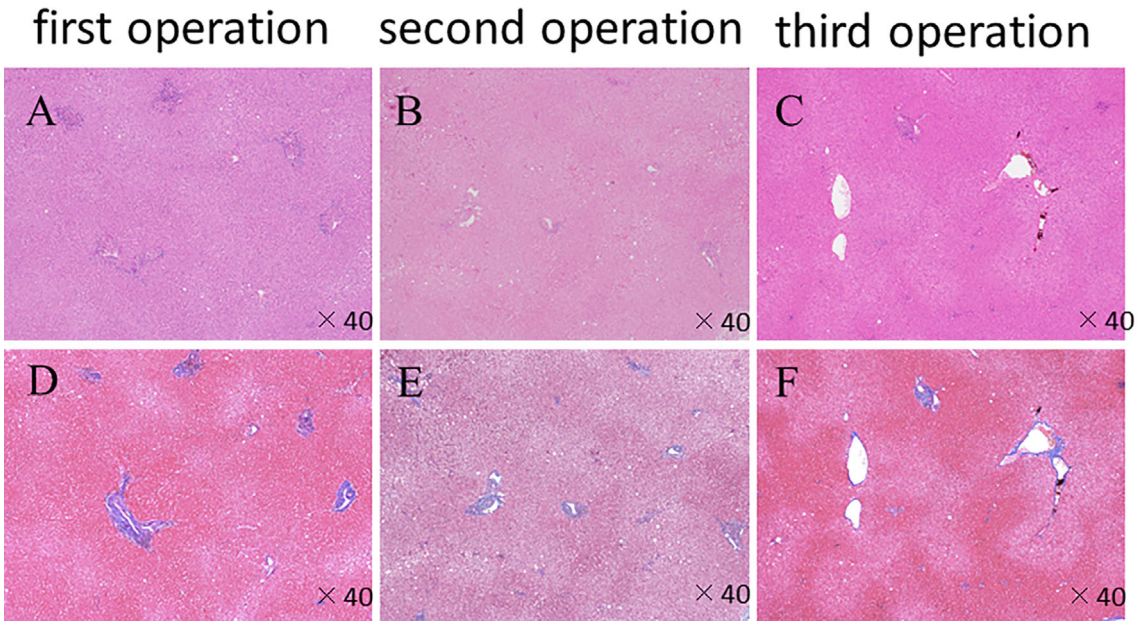

Figure 3. **Histopathological findings of the non-tumor areas of the resected specimens A, B, C; Hematoxylin and Eosin staining ×40. D, E, F; Masson's Trichrome stain ×40. All specimens showed <3% steatosis and mild fibrosis (Fibrosis 1).**

trahepatic metastases (10, 11).

The pathomechanisms involved in HCC development in this patient are not clear at present. A recent study of 2,087 Japanese patients with HCC indicated that non-viral etiologies (non-B non-C) increased from 10.0% in 1991 to 32.5% in 2015. The significant risk factors for HCC in these patients were old age, obesity, diabetes mellitus, hypertension, dyslipidemia, and fatty liver (1). In our patient with a non-viral etiology, the potential risk factors for HCC are age and a history of fatty liver. Our patient also had a history of HBV infection, and thus a direct oncogenic role of HBV on HCC cannot be ruled out. Bralet et al. (5) retrospectively analyzed 80 patients in whom the non-tumor liver tissue samples showed no or minimal portal fibrosis without any septal fibrosis. The same study concluded that in most case, the etiologic factors were unidentifiable, although presence of HBV infection in 21% suggested a direct oncogenic role of this virus (5). Thus, the integration of HBV into host DNA might result in a dysregulated expression of the genes involved in cell cycle regulation (3, 12, 13). Further studies of patients who develop HCC with mild fibrosis are needed to investigate the mechanism of HCC development.

In this case, AFP and PIVKAII, which are tumor markers of HCC, were not useful for the early prediction of HCC. The long-term follow-up demonstrated the clinical usefulness of analyzing TERT C228T in the serum cfDNA for the early prediction of HCC (9). The limitations associated with the present study include the lack of an analysis of the relationships between serum TERT C228T and the curability of HCC, and between TERT C228T in the serum and HCC tissue. Especially, a *TERT* mutation in the serum after the curative resection of HCC should be investigated. In this case, at several points after triplet partial hepatectomy, TERT C228T was confirmed to be positive (data not shown). Regrettably, it was not regularly measured after resection every month, so we could not determine whether a

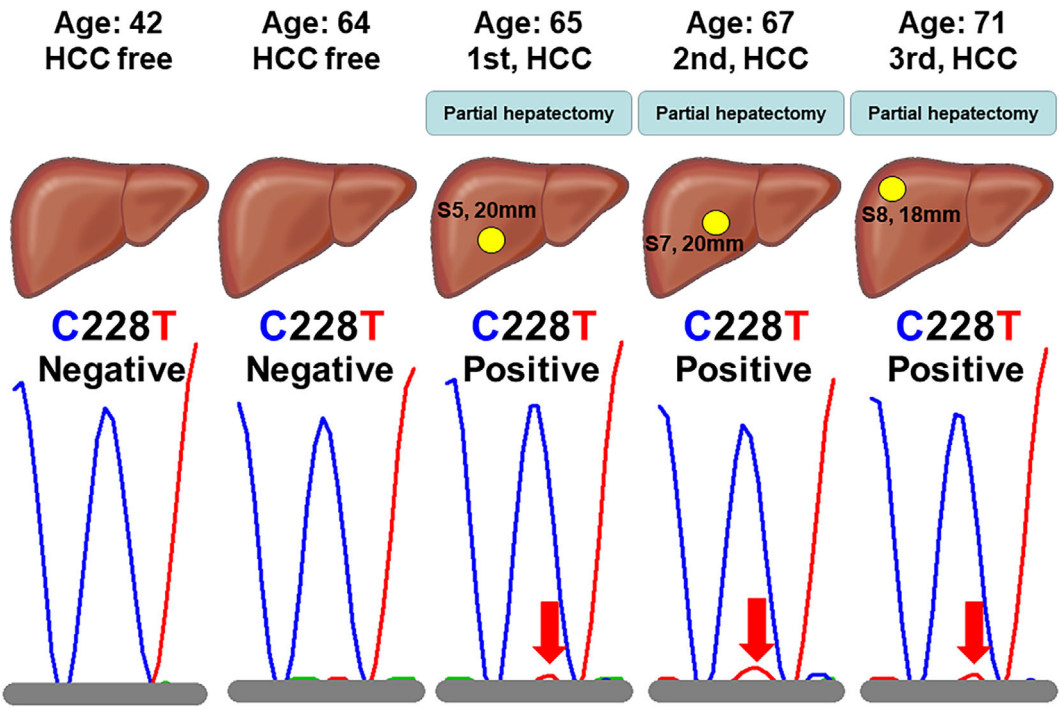

Figure 4. **Relationship between hepatocellular carcinoma (HCC) and TERT C228T in the serum cell-free DNA, examined serially using wild-type blocking PCR. TERT C228T was negative before the diagnosis of HCC at 64 years of age, but it became positive at the time that HCC was diagnosed at 65 years of age. The wild peak at position 228 (228C) is shown as the blue peak. The mutant peak at position 228 (228T) (red peak) detected at frequency of 0.7% or higher (Positive), but not at 0.6% (Negative). Three red arrows indicated the red mutant peak.**

positive TERT C228T finding might have continued regardless of the curability of HCC. This finding might suggest the existence of subclinical HCC. However, the clinicopathological data in Table showed that the first operation was curative with a negative surgical margin and no vascular invasion. The following HCCs, which may have existed at the time of the first operation, were not detected on mage findings with abdominal US, dynamic CT, and/or EOB-MRI. Apart from the fact that we only described a single patient, another limitation associated with the present study is the lack of an analysis of TERT C250T mutations, as another hotspot of *TERT* promoter mutations (14). New WTB-PCR-based highly-sensitive methods for the detection of *TERT* promotor mutation, including both TERT C228T and C250T, should therefore be developed in the future. Further studies of a large number of patients should be performed to investigate the association between *TERT* promotor mutation and HCC arising from non-B non-C. In addition, it is true that there are HCCs that are not mediated by *TERT* promotor mutation (14), and the development of an early diagnostic modality for such cases is also expected.

To the best of our knowledge, this is the first report on the importance of a long-term follow-up in non-B non-C chronic liver damage, regardless of the fibrosis stage. The measurement of *TERT* promoter mutations in the serum cfDNA was useful for the early prediction of HCC. The proportion of HCC patients with non-viral etiologies has continued to rise in recent years and the report of further cases will make it possible to achieve a better understanding of this type of HCC.

Author's disclosure of potential Conflicts of Interest (COI).
Norio Akuta: Honoraria, AbbVie and Gilead Sciences. Masahiro Kobayashi: Honoraria, Eisai. Yasuji Arase: Honoraria, AbbVie. Hiromitsu Kumada: Honoraria, MSD, Gilead Sciences, AbbVie, Eisai and Dainippon Sumitomo Pharma.

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