Table 2 Glucose-related characteristics of the study participants by a combination of GGT and ALT

	Men ($n = 545$)				Women ($n = 465$)			
	Both-low GGT <34 U/L	ALT-high	GGT-high GGT ≥34 U/L	Both-high	Both-low GGT <20 U/L	ALT-high	GGT-high GGT ≥20 U/L	Both-high
	ALT <23 U/L	ALT ≥23 U/L	ALT <23 U/L	ALT ≥23 U/L	ALT <16 U/L	ALT ≥16 U/L	ALT <16 U/L	ALT ≥16 U/L
n	199	75	79	193	164	72	70	159
FPG, mg/dL†	100 (24)	101 (19)	104 (22)	106 (23)**	93 (13)	92 (8)	96 (11)	101 (25)**
HbA1c, NGSP, %†	5.2 (0.6)	5.2 (0.7)	5.3 (0.6)	5.4 (0.8)*	5.1 (0.5)	5.1 (0.4)	5.1 (0.4)	5.4 (0.8)**
Fasting insulin μU/mL†	6.7 (5.4)	8.0 (4.9)*	7.4 (3.7)	11.6 (6.0)**	5.6 (2.3)	5.8 (2.6)	6.6 (3.2)	8.0 (4.6)**
HOMA-β†	112 (39)	113 (42)	102 (33)	101 (44)	76 (41)	76 (38)	76 (36)	101 (160)*
HOMA-IR†	1.5 (1.2)	1.8 (1.2)	1.8 (1.3)	2.8 (1.6)**	1.3 (0.6)	1.3 (0.6)	1.6 (0.9)	2.0 (1.4)**
Insulinogenic index†	0.88 (0.91)	1.24 (1.40)	0.70 (1.18)	1.06 (0.97)	1.2 (2.2)	1.5 (3.6)	0.8 (1.9)	1.2 (1.5)
OGTT pattern								
NGT‡	137 (68.8%)	50 (66.7%)	46 (58.2%)	96 (49.7%)**	129 (78.7%)	61 (84.7%)	53 (75.7%)	99 (62.3%)*
IGT‡	42 (21.1%)	16 (21.3%)	22 (27.8%)	62 (32.1%)*	32 (19.5%)	9 (12.5%)	13 (18.6%)	42 (26.4%)*
DM‡	20 (10.1%)	9 (12.0%)	11 (13.9%)	35 (18.1%)*	3 (1.8%)	2 (2.8%)	4 (5.7%)	18 (11.3%)**

Non-parametric procedures were employed for the analysis of background features of the patients in the ALT-high, GGT-high and both-high groups compared with the both-low group, including the Mann–Whitney *U*-test or Fisher's exact test. *$P < 0.01$ vs both-low. **$P < 0.001$ vs both-low.
†Data are mean (standard deviation). ‡Data are number of subjects (%).
ALT, alanine aminotransferase; DI, disposition index; DM, diabetes mellitus; FPG, fasting plasma glucose; GGT, γ-glutamyltransferase; HbA1c; hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; HOMA-β, Homeostasis Model of Assessment – β-cell function; HOMA-IR, Homeostasis Model of Assessment – Insulin Resistance; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; NGSP, National Glycohaemoglobin Standardization Program.

Table 3 Spearman's correlation coefficients for the relationship between liver enzymes and glycemic, insulin resistance and insulin secretion parameters

	Men		Women	
	ALT	GGT	ALT	GGT
ALT	–	0.548**	–	0.510**
GGT	0.548**	–	0.510**	–
BMI	0.426**	0.410**	0.151*	0.194**
FPG	0.103*	0.237**	0.156*	0.237**
HbA1c	0.041	0.051	0.092*	0.166**
HOMA-β	0.305**	0.109*	0.136**	0.089*
HOMA-IR	0.446**	0.409**	0.259**	0.261**
Insulinogenic index	0.142**	0.023	0.076	−0.074

*$P < 0.05$, ** $P < 0.001$.
ALT, alanine aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase; HbA1c, hemoglobin A1c; HOMA-β, Homeostasis Model of Assessment – β-cell function; HOMA-IR, Homeostasis Model of Assessment – Insulin Resistance.

Table 5. Multivariate analysis shows that the IGI of less than 0.4 in men occurred when a patient had high FPG of 100 mg/dL or more (odds ratio, 2.48; 95% CI, 1.57–3.91; $P < 0.001$) and high HbA1c of 5.7% or more (odds ratio, 2.83; 95% CI, 1.83–4.37; $P < 0.001$). The IGI of less than 0.4 in women occurred when the patient had high FPG of 100 mg/dL or more (odds ratio, 2.70; 95% CI, 1.61–4.52; $P < 0.001$) and high HbA1c of 5.7% or more (odds ratio, 2.23; 95% CI, 1.33–3.75; $P = 0.002$).

DISCUSSION

THE IMPACT OF joint association of ALT and GGT on IR in men and women with normal ranges of AST, ALT and GGT has been described in the present study. The insulin dynamics, such as IR and IGI, was evaluated based on the difference of median level of ALT and GGT. As shown in Table 1, ALT and GGT provided different values based on sex difference. Thus, we investigated the data according to the sex separately. The strengths of the present study are an evaluation of insulin dynamics in the large numbers of patients included.

The present study shows several findings with regard to the relationship between liver enzyme and glycemic state in Japanese healthy patients. First, FPG and HbA1c levels were higher in the both-high group than in the both-low group in both men and women. However, FPG and HbA1c levels in the ALT-high or GGT-high groups was not statistically higher than those in the both-low group. Second, plasma levels of glucose at 0–180 min after oral glucose loading were higher in the both-high group than in the both-low group in both men and women. Third, HOMA-IR in the both-high group was higher than that in the both-low group regardless of presence or absence of fatty liver. However, HOMA-IR in the ALT-high or GGT-high groups was not higher than that in the both-low group. These results suggest that combination of ALT and GGT is useful for evaluating the glucose state and IR compared with ALT or GGT only in patients with or without fatty liver. Fourth, the incidence of fatty liver in the both-high group was higher than that in the both-low group. Thus, it is probable that fatty liver enhances the liver enzyme and IR. Finally, the prevalence of IGI of less than 0.4 insulin secretion was not different among the four groups classified on the basis of median values for ALT and GGT. This result suggests that combination of ALT

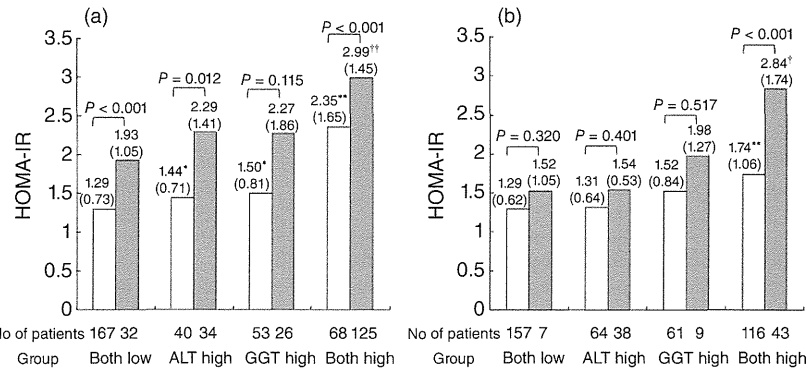

Figure 2 Homeostasis Model of Assessment – Insulin Resistance (HOMA-IR) based on the difference of liver enzymes and presence or absence of fatty liver. □, fatty infiltration (−); ■, fatty infiltration (+). ALT, alanine aminotransferase; GGT, γ-glutamyltransferase.

*P<0.05 vs both low without fatty liver, **P<0.001 vs both low without fatty liver, †P<0.05 vs both low with fatty liver, ††P<0.001 vs both low with fatty liver

Table 4 Predictive factors for elevated level of HOMA-IR

Variables	Univariate analysis Risk ratio (95% CI)	*P*	Multivariate analysis Risk ratio (95% CI)	*P*
Men				
Age (years, per 1)	1.01 (0.99–1.02)	0.756		
BMI (kg/m^2, per 5)	3.60 (2.60–4.98)	<0.001	1.57 (1.07–2.41)	0.042
Hypertension (+/−)	2.50 (1.46–4.26)	0.001		
AST (IU/L, ≥23/<23)*	3.31 (2.15–5.09)	<0.001		
ALT (IU/L, ≥23/<23)*	5.13 (3.30–8.00)	<0.001		
GGT (IU/L, ≥34/<34)*	4.88 (3.13–7.60)	<0.001		
Association of ALT and GGT (both-high/ALT-high or GGT-high/both-low)	3.65 (2.72–4.88)	<0.001	2.51 (1.68–3.75)	<0.001
FPG (mg/dL, ≥100/<100)	5.60 (3.60–8.69)	<0.001	5.23 (3.08–8.86)	<0.001
HbA1c (NGSP, %, ≥5.7/<5.7)	2.62 (1.77–3.90)	<0.001		
Albumin (g/dL, ≥4.0/<4.0)*	1.26 (0.84–1.88)	0.262		
Triglycerides (mg/dL, ≥105/<105)*	3.67 (2.40–5.62)	<0.001		
Total-C (mg/dL, ≥200/<200)*	1.45 (0.95–2.21)	0.084		
HDL-C (mg/dL, ≥46/<46)*	0.44 (0.29–0.68)	<0.001		
LDL-C (mg/dL, ≥126/<126)*	1.30 (0.85–2.00)	0.229		
Uric acid (mg/dL, ≥5.9/<5.9)*	1.60 (1.07–2.39)	0.022		
Platelets (×10^4/mm^3, ≥21.0/<21.0)*	0.63 (0.43–0.94)	0.022		
Fatty liver (+/−)	6.03 (3.92–9.27)	<0.001	2.32 (1.33–4.03)	0.003
Ethanol (g/day, ≥20/<20)	1.38 (0.89–2.81)	0.156		
Exercise (MET h/day, ≥2/<2)	0.31 (0.21–0.47)	<0.001		
Current smoking (+/−)	0.87 (0.60–1.12)	0.676		
Women				
Age (years, per 1)	1.02 (0.99–1.05))	0.092		
BMI (kg/m^2, per 5)	3.31 (2.20–5.00)	<0.001	1.86 (1.11–3.10)	0.018
Hypertension (+/−)	3.15 (1.37–7.26)	0.007		
AST (IU/L, ≥21/<21)*	1.89 (1.04–3.47)	0.037		
ALT (IU/L, ≥16/<16)*	3.67 (2.05–6.53)	<0.001		
GGT (IU/L, ≥20/<20)*	3.93 (2.12–7.27)	<0.001		
Association of ALT and GGT (both-high/ALT-high or GGT-high/both-low)	3.07 (2.05–4.60)	<0.001	2.21 (1.41–3.44)	<0.001
FPG (mg/dL, ≥100/<100)	11.17 (6.05–20.61)	<0.001	7.93 (4.11–15.29)	<0.001
HbA1c (NGSP, %, ≥5.7/<5.7)	3.74 (2.15–6.51)	<0.001		
Albumin (g/dL, ≥3.9/<3.9)*	1.79 (0.94–3.42)	0.077		
Triglycerides (mg/dL, ≥80/<80)*	2.23 (1.25–3.97)	0.006		
Total-C (mg/dL, ≥209/<209)*	1.09 (0.63–1.87)	0.267		
HDL-C (mg/dL, ≥61/<61)*	0.34 (0.19–0.60)	<0.001		
LDL-C (mg/dL, ≥128/<128)	1.25 (0.72–2.15)	0.432		
Uric acid (mg/dL, ≥4.6/<4.6)*	2.13 (1.19–3.78)	<0.001		
Platelets (×10^4/mm^3, ≥22.0/<22.0)*	0.58 (0.32–1.03)	0.065		
Fatty liver (+/−)	9.51 (5.24–17.24)	<0.001	3.80 (1.73–8.32)	0.001
Ethanol (g/day, ≥20/<20)	1.63 (0.80–3.34)	0.182		
Exercise (MET h/day, ≥2/<2)	0.48 (0.27–0.85)	0.011		
Current smoking (+/−)	0.812 (0.37–1.77)	0.584		

*These factors were divided into two groups by median value.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DI, disposition index; CI, confidence interval; DM, diabetes mellitus; FPG, fasting plasma glucose; GGT, γ-glutamyltransferase; HbA1c; hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; HOMA-β, Homeostasis Model of Assessment – β-cell function; HOMA-IR, Homeostasis Model of Assessment – Insulin Resistance; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; NGSP, National Glycohaemoglobin Standardization Program; Total-C, total cholesterol.

Table 5 Predictive factors for elevated level of IGI

Variables	Univariate analysis Risk Ratio (95% CI)	*P*	Multivariate analysis Risk Ratio (95% CI)	*P*
Men				
Age (years, per 1)	1.03 (1.01–1.05)	0.002		
BMI (kg/m^2, per 5)	0.85 (0.54–1.20)	0.101		
Hypertension (+/−)	1.02 (0.59–1.78)	0.943		
AST (IU/L, ≥23/<23)*	0.72 (0.50–1.04)	0.083		
ALT (IU/L, ≥23/<23)*	0.53 (0.36–0.77)	0.001		
GGT (IU/L, ≥34/<34)*	0.60 (0.41–0.88)	0.008		
Association of ALT and GGT (both-high/ALT-high or GGT-high/both-low)	0.91 (0.85–0.97)	0.068		
FPG (mg/dL, ≥100/<100)	2.81 (1.91–4.14)	<0.001	2.48 (1.57—3.91)	<0.001
HbA1c (NGSP, %, ≥5.7/<5.7)	2.62 (1.77–3.90)	<0.001	2.83 (1.83–4.37)	<0.001
Albumin (g/dL, ≥4.0/<4.0)*	0.75 (0.33–1.71)	0.478		
Triglycerides (mg/dL, ≥105/<105)*	0.67 (0.46–0.97)	0.035		
Total-C (mg/dL, ≥200/<200)*	0.84 (0.58–1.22)	0.358		
HDL-C (mg/dL, ≥46/<46)*	1.14 (0.78–1.68)	0.492		
LDL-C (mg/dL, ≥126/<126)*	0.93 (0.61–1.43)	0.739		
Uric acid (mg/dL, ≥5.9/<5.9)*	0.83 (0.71–0.96)	0.013		
Platelets (×10^4/mm^3, ≥21.0/<21.0)*	0.79 (0.54–1.15)	0.226		
Fatty liver (+/−)	0.78 (0.53–1.15	0.207		
Ethanol (g/day, ≥20/<20)	1.12 (0.74–1.68)	0.599		
Exercise (MET h/day, ≥2/<2)	1.10 (0.76–1.59)	0.625		
Current smoking (+/−)	0.98 (0.67–1.42)	0.975		
Women				
Age (year, per 1)	1.02 (1.00–1.05)	0.019		
BMI (kg/m^2, per 5)	0.91 (0.68–1.26)	0.914		
Hypertension (+/−)	1.41 (0.79–2.77)	0.359		
AST (IU/L, ≥21/<21)*	1.08 (0.69–1.72)	0.724		
ALT (IU/L, ≥16/<16)*	1.03 (0.62–1.66)	0.928		
GGT (IU/L, ≥20/<20)*	1.62 (0.84–2.58)	0.150		
Association of ALT and GGT (both-high/ALT-high or GGT-high/both-low)	1.07 (0.97–1.18)	0.128		
FPG (mg/dL, ≥100/<100)	3.58 (2.21–5.79)	<0.001	2.70 (1.61–4.52)	<0.001
HbA1c (NGSP, %, ≥5.7/<5.7)	3.05 (2.06–4.49)	<0.001	2.23 (1.33–3.75)	0.002
Albumin (g/dL, ≥3.9/<3.9)*	0.92 (0.57–1.50)	0.742		
Triglycerides (mg/dL, ≥80/<80)*	0.84 (0.54–1.34)	0.474		
Total-C (mg/dL, ≥209/<209)*	0.99 (0.63–1.57)	0.986		
HDL-C (mg/dL, ≥61/<61)*	0.82 (0.52–1.30)	0.393		
LDL-C (mg/dL, ≥128/<128)*	0.81 (0.50–1.29)	0.369		
Uric acid (mg/dL, ≥4.6/<4.6)*	1.12 (0.70–1.76)	0.652		
Platelets (×10^4/mm^3, ≥22.0/<22.0)*	0.87 (0.55–1.39)	0.569		
Fatty liver (+/−)	1.64 (0.92–2.95))	0.096		
Ethanol (g/day, ≥20/<20)	0.70 (0.33–1.48)	0.352		
Exercise (MET h/day, ≥2/<2)	0.81 (0.58–1.15)	0.240		
Current smoking (+/−)	1.43 (0.80–2.59)	0.229		

*These factors were divided into two groups by median value.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DI, disposition index; CI, confidence interval; DM, diabetes mellitus; FPG, fasting plasma glucose; GGT, γ-glutamyltransferase; HbA1c; hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; HOMA-β, Homeostasis Model of Assessment – β-cell function; HOMA-IR, Homeostasis Model of Assessment – Insulin Resistance; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; NGSP, National Glycohaemoglobin Standardization Program; Total-C, total cholesterol.

and GGT is ineffective for evaluating the insulin secretion insufficiency. Taken together, men with ALT of 23 IU/L or more and GGT of 34 IU/L or more, or women with ALT of 16 IU/L or more and GGT of 20 IU/L or more, tend to have IR and fatty liver. These results suggest that the combination of ALT and GGT is useful for the detection of IR, glucose tolerance and the presence of fatty liver. Even when the liver enzymes of ALT and GGT are within normal limits, the subjects with liver enzymes of median values or higher should be regularly checked for preventing the onset of T2DM, fatty liver or metabolic disease in future.

It has been reported that aminotransferase and GGT levels independently predict T2DM,[27–31] metabolic syndrome[32,33] and cerebrovascular disease.[29] These markers have been shown to be associated with indirect measurements of IR including fasting insulin levels and HOMA-IR.[33] In the present study, our data suggest that the joint association of ALT and GGT could be useful for predicting IR compared to ALT or GGT only.

Although the role of higher ALT levels for the risk of T2DM is not clear, the following possible mechanism have been reported: (i) ALT level may be a marker of visceral fat deposits in the liver and thus reflect the status of metabolic fatty liver disease and IR;[34–36] and (ii) ALT level is correlated with subclinical systemic inflammation, increased oxidative stress and higher hepatic cytokine production, such as C-reactive protein and tumor necrosis factor-α. These factors may cause metabolic abnormalities, such as impaired insulin signaling in the liver and other organs, and increase the risk for the development of T2DM.[9,37] Similarly, the following possible mechanism of elevated GGT in the pathogenesis of T2DM have been reported: (i) GGT is related to IR by meta-analysis;[38–40] and (ii) GGT is linked with systemic low-grade inflammation and a sensitive marker of oxidative stress.[41,42] These factors may enhance the development of T2DM.

The prevalence of T2DM has been increasing dramatically worldwide for the past decades. Various factors have been reported as risk factors for the development of T2DM,[2–4] and a recent meta-analysis suggested that GGT may be a better predictor for T2DM than ALT, but the authors also reported that further studies with directly determined liver fat content, ALT and GGT are needed to confirm the association.[43] Our study results suggest that the joint association of ALT and GGT is valuable for evaluating glycemic state compared with ALT or GGT only. Combining the two liver function markers would be effective for identifying individuals experiencing deterioration of glucose tolerance and IR, namely, those who are at high risk of the development of diabetes.

The present study has several limitations. First, although viral hepatitis was excluded, medication, iron overload and other causes that we could not exclude in the present study might have affected the liver enzyme levels of our participants. Second, most of the patients did not undergo histological or morphological assessment by peritoneoscopy or liver biopsy. Third, most of the women were not evaluated for menopausal status and hormone replacement treatment. In addition, waist circumference was measured in some individuals before 2007 in our health management center. Thus, waist circumference was not examined in the present study. Finally, our cohort contains Japanese subjects only.

In conclusion, combining the two liver function markers of ALT and GGT would be effective for identifying individuals with deterioration of glucose tolerance or IR, namely, those who are at high risk of the development of diabetes.

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ORIGINAL SCIENTIFIC REPORT

The Influence of Histological Differentiation Grade on the Outcome of Liver Resection for Hepatocellular Carcinomas 2 cm or Smaller in Size

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Abstract

Background Small hepatocellular carcinomas (HCC) with poorly differentiated components (PDC) are reportedly at risk of dissemination and needle tract seeding after percutaneous radiofrequency ablation, although it is the preferred treatment for HCC $\leq$ 2 cm because of the low rate of vascular invasion. On the other hand, the clinical outcomes after hepatectomy for these tumors are still unclear because of their rarity.

Methods A total of 233 cases of solitary HCC $\leq$ 2 cm were retrospectively reviewed and divided into two groups according to the presence of PDC: 199 without PDC (NP-HCCs) and 34 with PDC (P-HCCs). The clinicopathological characteristics and prognosis were compared.

Results A comparison of clinicopathological characteristics showed that the elevation of the tumor markers alpha-fetoprotein (AFP) (>20 ng/mL) and des-gamma-carboxyprothrombin (DCP) (>40 AU/L) was significantly frequent in P-HCCs. The 3- and 5-year recurrence-free survival rates for P-HCCS were 39 and 29 %, respectively, which were significantly worse than those for NP-HCCs (64 and 50 %, respectively) ($p < 0.01$). Initial recurrence of P-HCCs was significantly more frequent, as well as extrahepatic recurrence and advanced recurrence in the early period after the operation. Recurrences with tumor dissemination were observed in 15 % of P-HCCs and 4 % of NP-HCCs ($p = 0.03$).

Conclusion PDC is present in 15 % of HCC < 2 cm and should be suspected when the both tumor markers are elevated. Moreover, significantly worse post-hepatectomy outcomes such as early advanced recurrence or recurrence with dissemination should be taken into account if PDC is present even in HCCs $\leq$ 2 cm.

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Introduction

Classic hepatocellular carcinoma (HCC) is known to develop in a multistep fashion. Well-differentiated areas are gradually replaced by less well-differentiated tissue when the tumor size reaches a diameter of approximately 2 cm [1, 2]. It is now possible to detect small HCCs because of advances in imaging and establishment of screening guidelines for patients with a high risk of HCC, resulting in an increasing number of early HCC that

undergo treatment [3]. In these situations, surgeons may encounter cases of resected HCCs of a small size (2 cm or smaller) that have poorly differentiated components (PDC). The carcinogenesis process of these tumors deviates greatly from the well-known multistep development theory.

The HCCs that have PDCs are reportedly at risk of dissemination, needle tract tumor seeding, and intrahepatic dissemination after percutaneous radiofrequency ablation (RFA). Based on these observations, several reports recommend hepatectomy rather than RFA for HCCs that contain PDCs and pre-treatment detection of PDCs in HCCs has attracted attention [4–6]. However, the influence of PDCs in small HCCs on outcomes after hepatectomy and the characteristics of these tumors remain unclear.

The aim of the present study was to examine the clinicopathological characteristics of small HCCs $\leq$ 2 cm with PDC, and to determine whether the outcome of hepatectomy is influenced by the histologic grade in small HCCs $\leq$ 2 cm.

Methods

Patients

A total of 649 cases that underwent primary curative hepatectomy for HCC (R0 or R1 resection) between 1994 and 2012 were retrospectively reviewed. Of the 649 cases, 246 had solitary tumors $\leq$2 cm in size and were examined in detail. Cases with solitary tumors were chosen to enable accurate assessment of the influence of histologic grade on operative outcomes. Ten cases with an uncertain histologic grade due to total necrosis secondary to preoperative transcatheter chemoembolization were excluded, as well as three cases with unclear outcomes 1 year after primary resection. Thus, 233 cases of solitary HCC $\leq$ 2 cm were analyzed in this study, which were followed up until death or December 2013. The clinicopathological characteristics of these cases are shown in Table 1, including the number and size of tumors, width of the surgical margin, and presence of liver cirrhosis.

Table 1 Comparison of clinicopathological characteristics between differentiation grades

Differentiation grade[a]	Non-poorly		Poorly $N = 34$	Non-poorly versus poorly
	Well $N = 54$	Moderately $N = 145$		
(a) Patient-related factors				
Male sex % (yes/no)	**72** (39/15)	**75** (109/36)	**79** (27/7)	0.67
Age > 60 years % (yes/no)	**67** (36/18)	**48** (70/75)	**53** (18/16)	1.00
HBV infection positive % (yes/no)	**22** (12/42)	**36** (52/93)	**29** (10/24)	0.84
HCV infection positive % (yes/no)	**70** (38/16)	**57** (82/63)	**62** (21/13)	1.00
(b) Background liver-related factors				
Platelet count $<10^5$ (μL) % (yes/no)	**46** (25/29)	**34** (49/96)	**35** (12/22)	1.00
Child-Pugh grade B % (yes/no)	**22** (12/42)	**8** (12/133)	**12** (4/30)	1.00
Liver cirrhosis % (yes/no)	**70** (38/16)	**66** (95/50)	**79** (27/7)	0.17
(c) Pre-operative tumor markers				
AFP > 100 ng/mL % (yes/no)	**11** (6/48)	**20** (29/116)	**26** (9/25)	0.24
DCP > 100 AU/L %[b] (yes/no)	**2** (1/51)	**12** (17/123)	**15** (5/28)	0.35
Elevation of both tumor markers[b,c] (yes/no)	**4** (2/50)	**10** (14/126)	**27** (9/24)	*<0.01*
(d) Operative factors				
Anatomical resection % (yes/no)	**5** (2/52)	**17** (24/121)	**24** (8/26)	0.12
Surgical margin >5 mm % (yes/no)	**46** (25/29)	**54** (78/67)	**47** (16/18)	0.71
(e) Tumor-related factors				
Non-boundary macroscopic appearance % (yes/no)	**22** (12/42)	**39** (56/89)	**47** (16/18)	0.18
Capsule formation % (yes/no)	**37** (20/34)	**72** (104/41)	**71** (24/10)	0.44
Microscopic vascular invasion % (yes/no)	**9** (5/49)	**18** (26/119)	**24** (8/26)	0.32

HBV hepatitis B virus, *HCV* hepatitis C virus, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxyprothrombin

[a] Differentiation grade was determined by the differentiation component with the worst grade in the entire specimen

[b] DCP was not measured in six patients

[c] Concurrent elevation of alpha-fetoprotein (>20 ng/mL) and des-gamma-carboxyprothrombin (>40 AU/L)

Treatment selection

The indications for hepatectomy were basically compatible with the recommendations of the Consensus-Based Clinical Practice Manual proposed by the Japan Society of Hepatology [7]. In our institution, local ablation such as RFA is generally recommended for solitary HCCs $\leq$ 2 cm. Local ablation treatments as curative treatment had been indicated in 352 cases of HCCs $\leq$ 2 cm during the study period. Hepatectomy was indicated for cases with preserved liver function with the HCC located peripherally, in the subphrenic space, or near major vasculature. Anatomical resection (AR) was defined as resection of liver segments according to the territories supplied by the portal vein. AR was performed for tumors located centrally or close to major vessels if appropriate, while limited non-AR was preferred for tumors located peripherally or if extrahepatic growths were present. In addition, AR was indicated for tumors with a high risk of spread such as those with "a heterogeneous enhancement pattern with irregular ring-like structures" on three-phase-enhanced CT, and high tumor marker levels, regardless of tumor size [8]. The width of the surgical margin was principally 5 mm because previous studies showed that small tumors seldom have microsatellite lesions greater than 5 mm [9–11]. None of the patients received liver transplantation for HCC $\leq$ 2 cm during the study period.

Histopathologic examination and definition of clinical variables

Two experienced pathologists examined the resected specimens. Variables were defined according to "the general rule for the clinical and pathological study of primary liver cancer" determined by the Liver Cancer Study Group of Japan, and the pathological classification system of the World Health Organization [12, 13]. PDC was defined according to the aforementioned pathological guidelines, as follows: "PDC proliferate in a solid pattern without distinct sinusoid-like blood spaces, and only slit-like blood vessels are observed in large tumor nests. Neoplastic cells show an increased nuclear/cytoplasmic ratio and frequent pleomorphism, including bizarre giant cells" [13]. Only four patients had micrometastases around the main tumor.

The macroscopic appearance of the HCC was divided into two groups based on the classification of the aforementioned guidelines: boundary type, which included the vaguely nodular and single nodular type, and non-boundary type, which included the single nodular with extranodular growth, the confluent multinodular, and the invasive type. Elevated levels of both tumor markers meant concurrent elevation of AFP > 20 ng/mL and DCP > 40 AU/L).

Postoperative follow-up and definition of recurrence pattern

Patients were followed up monthly while tumor markers were measured every month, ultrasonography was performed every 3 months, and dynamic CT or MRI was performed every 6 months for the first 2 years after the operation. After 2 years, the follow-up period was determined according to the likelihood of recurrence, but the imaging schedule remained principally the same. Additional imaging studies were performed if recurrence was suspected. Dynamic CT/MRI and/or CT angiography were used to determine the presence of multiple recurrent tumors. CT, MRI, and scintigraphy were used to detect extrahepatic recurrence. The site and pattern of the initial recurrence were defined as follows: (1) solitary recurrence; (2) oligonodular (two or three tumor nodules) recurrence; (3) recurrence with four or more lesions; and (4) recurrence at an extrahepatic site regardless of concurrent intrahepatic recurrence. In this study, "advanced recurrence" was defined as recurrence with four or more lesions and/or extrahepatic recurrence. Neither recurrence with macroscopic vascular invasion nor concurrent intra- and extrahepatic recurrence were observed in our study population.

Design

Of the total of 233 patients with HCC, 54 had well-differentiated HCC, 145 had moderately differentiated HCC, and 34 had poorly differentiated HCC (P-HCC). The 199 HCC patients without PDC were grouped together as cases of non-poorly differentiated HCC (NP-HCC). A retrospective comparison of variables in the NP-HCC and P-HCC groups were performed (Table 1), as well as pattern of recurrence, second treatments after initial recurrence, and cumulative recurrence-free survival rate.

Subgroup analyses were conducted to investigate the influence of the surgical approach, including AR and resection margin width ($\geq$5 mm or <5 mm), on the recurrence-free survival of patients with small P-HCC.

Statistical analysis and ethical considerations

The data were analyzed with SPSS software ver. 21 (IBM SPSS, Chicago, IL, USA). All variables were categorized and their values were expressed as percentages. The χ^2 or Fisher's exact test was used to compare categorical variables between two groups, as appropriate. Cumulative overall survival and recurrence-free survival were determined using the Kaplan–Meier method. Differences between curves were assessed using the generalized Wilcoxon test. Multivariate analysis with Cox stepwise regression was used to investigate independent predictors

of prognosis. In multivariate analyses, all factors included in Table 1 and the presence of PDC were entered into the analyses. A p value of <0.05 was considered statistically significant.

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital.

Results

Of the 233 patients, 175 were men and 58 were women. The median age at the time of hepatectomy was 61 years (range 35–79 years). The median tumor size was 16 mm (range 6–20 mm). There were two cases (0.9 %) of in-hospital mortality related to hepatectomy. A total of 132 patients (57 %) had recurrence and 79 patients died during the follow-up period. The median follow-up period for survivors was 69.0 months (range 5.1–239.6 months).

Clinicopathological characteristics of HCC ≤ 2 cm with PDC

The clinicopathological characteristics of tumors in each differentiation grade are shown in Table 1. Results showed that poor histologic differentiation was associated with an increased likelihood of elevated tumor markers, non-boundary macroscopic tumor appearance, and microscopic vascular invasion. Comparison of the NP-HCC and P-HCC groups revealed that concurrent elevation of the two tumor markers was the only significant difference between the two groups.

Recurrence patterns and second treatment according to the presence of PDC

Compared with NP-HCCs, P-HCCs were more likely to recur, extrahepatic recurrence was more common, and advanced recurrence in the early period after operation was more likely (Table 2). In cases of recurrence of P-HCC, four cases of advanced recurrence were of the disseminated type, and one case of extrahepatic recurrence had peritoneal dissemination (5/34, 15 %), on the other hand, recurrence with dissemination was seen in only eight cases of NP-HCCs (8/199, 4 %) ($p = 0.03$). The second treatments for initial recurrence are also shown in Table 2. TACE was significantly more frequent in the poorly differentiated group than in the non-poorly differentiated group. None of the patients received salvage liver transplantation for initial recurrence.

Table 2 Comparison of recurrence patterns and second treatment based on the presence of poorly differentiated component

	Non-poorly		Poorly $N = 34$	Non-poorly versus poorly
	Well $N = 54$	Moderately $N = 145$		
Overall recurrence (%)	26 (48)	80 (55)	26 (76)	*0.01*
Initial recurrence pattern (%)				
Solitary	23 (43)	52 (36)	13 (38)	1.00
Oligonodular	1 (2)	15 (10)	3 (9)	1.00
Four or more	2 (4)	13 (9)	6 (18)	*0.10*
Extrahepatic	0 (0)	0 (0)	4 (12)	*<0.01*
Initial recurrence pattern and time to recurrence (%)				
Recurrence within 1 year	4 (7)	18 (12)	8 (24)	0.06
Recurrence within 2 years	12 (22)	37 (26)	13 (38)	0.14
Advanced recurrence within 1 year[a]	0 (0)	4 (3)	8 (24)	*<0.01*
Advanced recurrence within 2 years[a]	0 (0)	10 (7)	8 (24)	*<0.01*
The second treatment after initial recurrence (%)				
Hepatectomy	5 (9)	21 (14)	5 (15)	1.00
RFA	15 (28)	27 (19)	6 (18)	0.66
TACE	5 (9)	29 (2)	12 (35)	*0.02*
Others	1 (2)	3 (2)	2 (6)	0.21

[a] Advanced recurrence was defined as recurrence with four or more lesions and/or extrahepatic recurrence

Comparison of prognosis based on the presence of PDC

The 5-year recurrence-free survival rate of all patients was 47 %. The recurrence-free survival curves for both groups are shown in Fig. 1. The 3-, 5-, and 10-year recurrence-free survival rates for NP-HCCs were 64, 50, and 32 % respectively, while those for P-HCCS were 39, 29, and 15 % respectively. The recurrence-free survival rates for P-HCC were significantly worse than those for NP-HCCs ($p < 0.01$).

Subgroup analyses were performed after stratifying each treatment approach by the presence of PDC (Fig. 2). In P-HCCs, AR resulted in a significantly lower recurrence rate compared to NAR ($p = 0.04$), although no significant difference was observed for NP-HCCs ($p = 0.73$). Additionally, in the analyses of resection margin width, a wider resection margin did not significantly reduce the recurrence rate compared with a narrower resection margin, regardless of the presence of PDC ($p = 0.32$, $p = 0.96$, respectively) (Fig. 3).

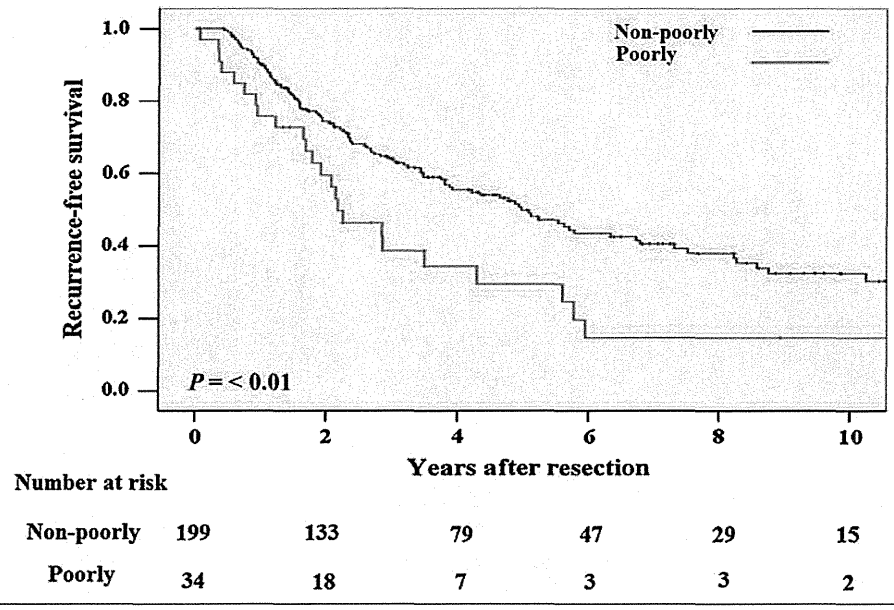

Fig. 1 The long-term prognostic impact of poorly differentiated components

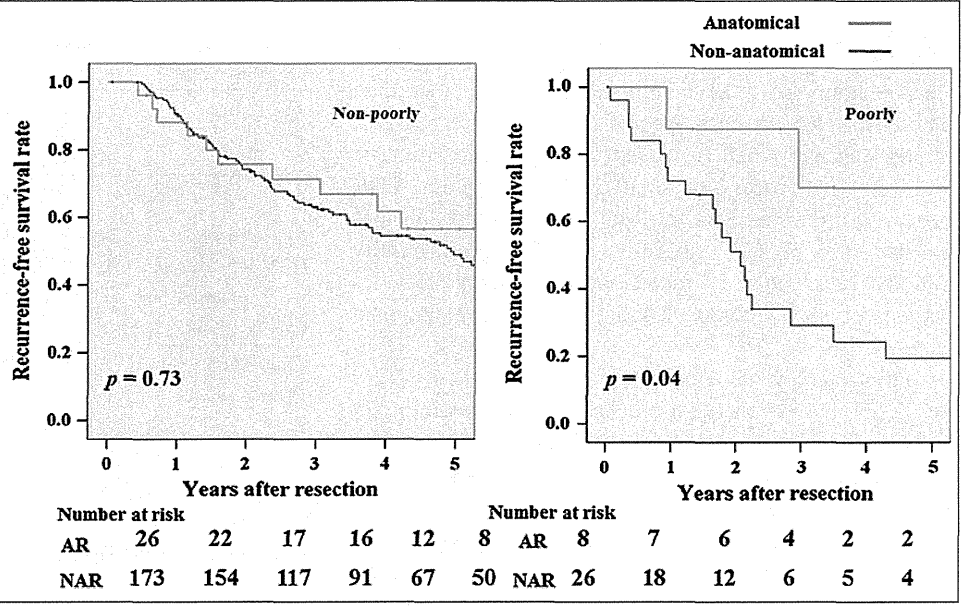

Fig. 2 The influence of surgical approach according to the presence of PDC. *PDC* poorly differentiated component *AR* anatomical resection, *NAR* non-anatomical resection

The results of univariate and multivariate analyses regarding the predictor of recurrence are shown in Table 3. In the univariate analysis, non-hepatitis B infection, hepatitis C infection, low serum platelet count, Child-Pugh grade B, high AFP level, the presence of PDC, and microscopic vascular invasion were significant predictors of recurrence. In the multivariate analysis, male sex, non-hepatitis B infection, low serum platelet count, high AFP level, and the presence of PDC were independent predictors of recurrence (Table 3). Both AR and wide resection margin were not independent predictors of recurrence.

Discussion

The existence of small HCCs with PDC has been recognized and their treatment has recently increased. However, little is known about the characteristics of these tumors because small tumors with PDC were considered to be a rare entity until recently. Additionally, these small HCC were considered as good candidates for ablation therapy and their pathological differentiation characteristics were not examined. In previous large-scale studies on hepatectomy for solitary HCC $\leq$ 2 cm, 6–23 % of patients had

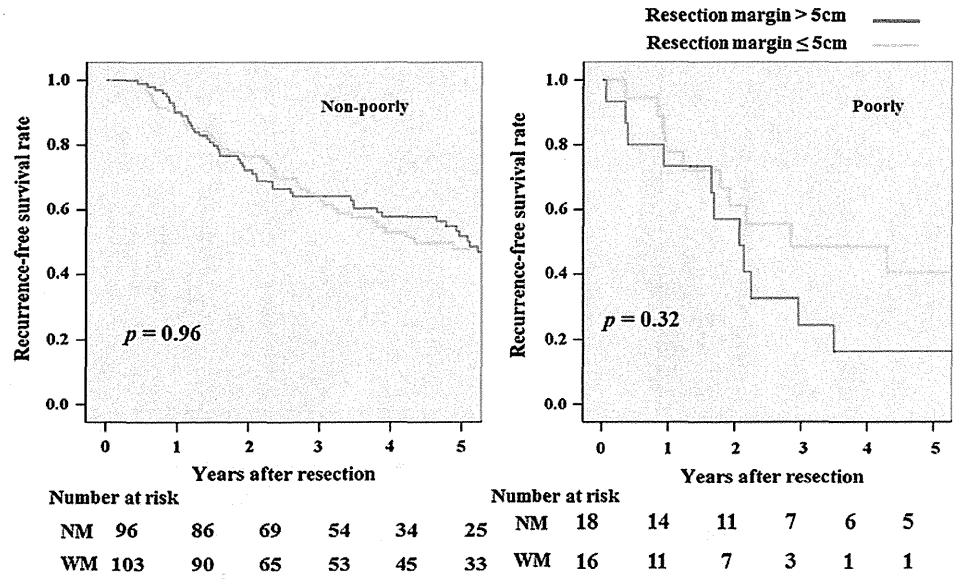

Fig. 3 The influence of the resection margin width on recurrence-free survival according to the presence of PDC. *NM* narrow margin, *WM* wide margin

PDC, which were compatible with our result [14–17]. Even if hepatectomy was the treatment of choice rather than ablation for HCCs with suspected invasive characteristics, the prevalence of small P-HCCs would not be so rare. The carcinogenesis process of small HCCs containing PDC appears to differ from the well-known multistep development theory of HCC. Further investigation into this entity from the perspective of molecular biology and genetics may enable a better understanding of the developmental process of HCC.

Current therapy guidelines do not recommend RFA for P-HCCs because of the poor outcome and the possibility of tumor seeding, moreover, P-HCCs has been considered to be a contraindication for liver transplantation in some institutions because of post-transplant recurrence and poor survival [18–20]. Therefore, the importance of preoperative detection of PDC in small HCCs has been increasingly emphasized, and recent advances in imaging modalities have made detection possible. The current study showed that elevation of both tumor markers in cases of very small solitary HCCs may be a useful preoperative predictor of the presence of PDC. Using the elevation of tumor markers to predict the presence of PDC is advantageous, especially for HCCs $\leq$ 2 cm, because imaging evaluation of small tumors remains inadequate.

The current study showed that the postoperative outcomes of P-HCCs were worse than those of NP-HCC. Univariate and multivariate analyses showed that the presence of PDC was a poor predictor of recurrence, and tumor recurrence with dissemination was observed in 15 % (5/34) of HCCs with PDC. Considering those postoperative

Table 3 The results of univariate and multivariate analysis regarding recurrence

	Univariate	Multivariate		
	P	*p*	HR	95 %CI
Male sex	0.12	<0.01	2.26	1.40–3.63
Age >60 years	0.48			
HBV infection positive	<0.01	<0.01	0.45	0.30–0.68
HCV infection positive	<0.01			
Platelet count $<10^5$ (μL)	<0.01	0.02	2.11	1.47–3.04
Child-Pugh grade B	0.04			
Liver cirrhosis	0.46			
AFP > 100 ng/mL	0.02	<0.01	1.92	1.23–3.00
DCP > 100 AU/L[b]	0.78			
Elevation of both tumor markers[b,c]	0.21			
Anatomical resection	0.34			
Surgical margin >5 mm	0.50			
The presence of PDC	<0.01	<0.01	1.99	1.25–3.16
Non-boundary macroscopic appearance)	0.23			
Capsule formation	0.28			
Microscopic vascular invasion	0.02			

HBV hepatitis B virus, *HCV* hepatitis C virus, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxyprothrombin

[a] Differentiation grade was determined by the differentiation component with the worst grade in the entire specimen

[b] DCP was not measured in six patients

[c] Concurrent elevation of alpha-fetoprotein (>20 ng/mL) and des-gamma-carboxyprothrombin (>40 AU/L)

outcomes, a refinement in the operative strategy for small HCCs with preoperative suspicion of PDC should be discussed. Our subgroup analyses indicated an interesting result, although the statistical validity was insufficient because the number of patients was too small and those results were speculative. The clinical impact of AR for HCC remains controversial, although various studies have shown a firm clinical benefit of AR [21–23]. Considering that a wider surgical margin did not improve postoperative recurrence, eradication of the primary tumor in addition to any micrometastases or microsatellite lesions along the portal tributaries, which are undetectable by light microscopy, may play a role in preventing recurrence caused by PDC. We speculate that performing AR regardless of the tumor size in cases with preoperatively suspected PDC might improve their poor outcomes. However, a large, prospective, randomized controlled study involving multiple institutes is necessary to determine the clinical benefit of AR in the treatment of small HCCs with PDC.

The poor outcome of hepatectomy for solitary HCCs $\leq$ 2 cm remains controversial. The results of previous three large-scale studies of hepatectomy for solitary HCC $\leq$ 2 cm are summarized in Table 4 [14–17]. One Western multicenter study showed that the presence of PDC was predictive of early recurrence but not recurrence-free survival. One study showed that the presence of microscopic vascular invasion was a predictor of recurrence but not of survival in univariate analysis, and another study showed that none of the tumor-related factors including the presence of PDC were associated with long-term survival. Although these studies did not focus on tumor differentiation grade, there was no consensus on the impact of PDC in HCC $\leq$ 2 cm. Differences in study outcomes are likely to be due to heterogeneity in etiology, liver function, and treatment selection. Another possible cause is the subjective evaluation of tumor differentiation grade, which is a limitation of our study. Currently, there is no standard, objective assessment of the presence of PDC. As a result, the histological grade is inconsistent between institutions. Several studies have demonstrated an association between tumor differentiation grade and protein or cytokine expression [24, 25]. Novel methods to assess tumor differentiation based on these studies are attractive; however, they are not yet fully established or tested in the clinical setting. Our concern about inter-institute uniformity would be resolved by establishing a simple and objective histological grading system.

Table 4 Summary of large-scale studies on hepatectomy for solitary HCC $\leq$ 2 cm

Authors	*N*	Study period	Etiology	LC (%)	P-HCC (%)	MVI (%)	SL	Main results regarding postoperative prognosis
Midorikawa et al. 2013	248	1982-2011	NA	52	6	21	NA	None of P-HCC, MVI, and SL were not predictor of survival in multivariate analysis
Yamashita et al. 2012	149	1990–2009	Hep B 13 % Hep C 80 % Others NA	55	24	29	3	MVI was a predictor of recurrence in univariate analysis No multivariate analysis was performed
Roayaie et al. 2013	132	1995–2010	Hep B 37 % Hep C 52 % Others 11 %	67	16	27	12	SL and LC were poor predictors of recurrence in multivariate analysis SL and low serum platelet were poor predictors of survival P-HCC was a predictor of recurrence within 2 years
Shindoh et al. 2013	155	1981–2011	Hep B 37 % Hep C 38 % Others NA	60	18	26	NA	MVI was not a poor predictor of survival No multivariate analysis was performed
Sasaki et al. 2013	233	1993–2012	Hep B 32 % Hep C 59 % Others 9 %	69	15	17	2	P-HCC was a poor predictor of recurrence in multivariate analysis Extrahepatic recurrence was significantly frequent in P-HCC Early advanced recurrence was significantly frequent in P-HCC 15 % of P-HCC had recurrence with dissemination even after hepatectomy

LC liver cirrhosis, *P-HCC* hepatocellular carcinoma with poorly differentiated component, *MVI* positive for microscopic vascular invasion, *SL* presence of satellite lesion, *Hep B* positive for hepatitis B surface antigen, *Hep C* positive for hepatitis C antibody, *NA* not available

The current study is the first study that examined the influence of poor histological differentiation grade in HCCs $\leq$ 2 cm, although it was a retrospective study with a relatively small sample size from a single center. A multicenter large-scale study is needed to confirm our finding. Moreover, our study did analyze the influence of liver cirrhosis because of the small number of patients. We have to analyze again after splitting the patients into cirrhotic and non-cirrhotic groups in the future to confirm our findings.

In conclusion, our result indicated that the presence of PDC should be considered in cases of small solitary HCCs $\leq$ 2 cm with an elevation of both tumor markers. Moreover, significantly worse post-hepatectomy outcomes such as early advanced recurrence or recurrence with dissemination should be taken into account if PDC is present even in HCCs $\leq$ 2 cm.

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VI 特　論

輸血用血液の肝炎対策
—血液スクリーニングの現況—

Strategy for the prevention of transfusion-transmitted hepatitis
—current state of blood donor screening test—

内田茂治

Key words：輸血後肝炎，NAT，遡及調査，人畜共通感染症

はじめに

我が国の輸血用血液は1960年代半ばまで，そのほとんどが売血血液により賄われており，その売血時代には輸血を受けた患者の約半数が輸血後肝炎を発症していたと報告されている[1]．その後の献血制度への切り替えや新たな検査の導入により，輸血後肝炎の発生頻度は徐々に低下してきた(**図1**)[2]．1981年1月から，その当時非A非B型肝炎(大部分がC型肝炎と考えられ

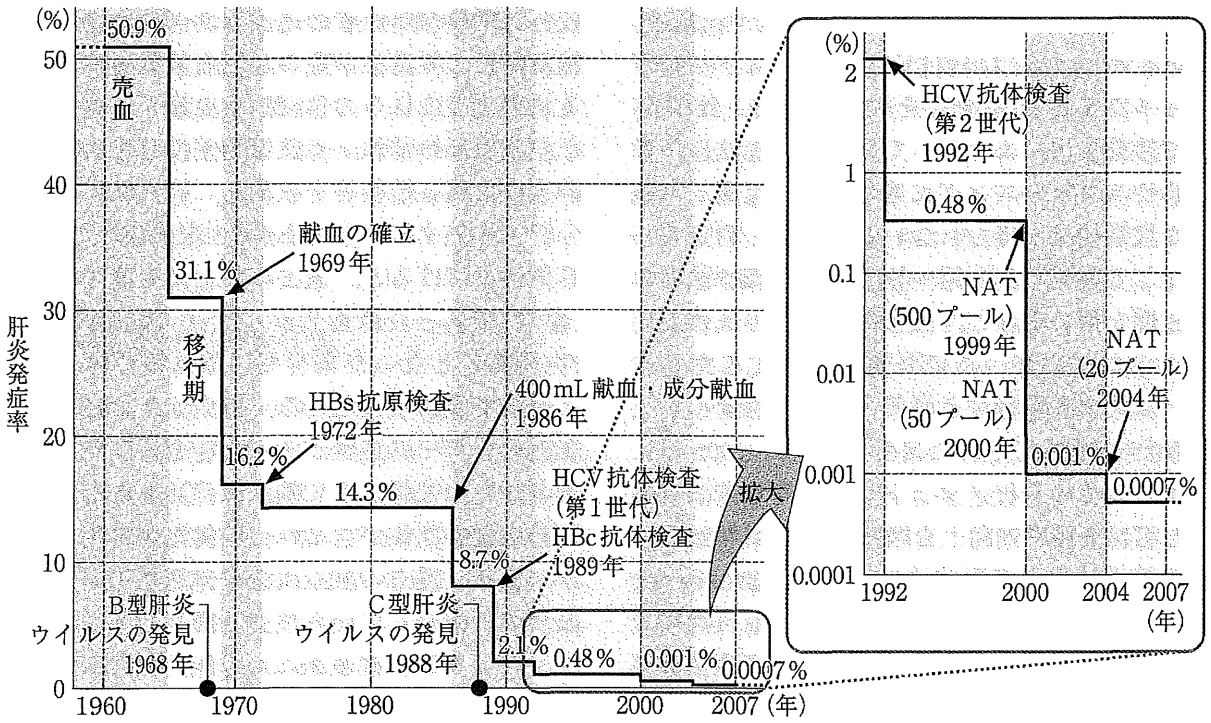

図1　我が国における輸血後肝炎の推移(輸血情報0811-116)

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0047-1852/15/¥60/頁/JCOPY

ている）と呼ばれていた肝炎対策としてGPT（ALT）検査が導入された．1989年12月にはB型肝炎ウイルス（hepatitis B virus: HBV）コア抗体（HBc抗体）検査，C型肝炎ウイルス（hepatitis C virus: HCV）抗体検査が導入され，1999年10月にはHBV，HCVおよびヒト免疫不全ウイルス（human immunodeficiency virus: HIV）を対象とした核酸増幅検査（nucleic acid amplification testing: NAT）が導入された．また，2014年8月からは個別検体によるNATを導入し，輸血用血液の安全性は着実に向上している．

本稿では，NAT導入後から現在に至るまでのスクリーニング検査の現況，輸血後肝炎発生状況ならびに各肝炎ウイルスに対する対策について述べる．

1 スクリーニング検査の変遷

献血された血液の感染症血清学的検査は，梅毒血清学的検査，HBV関連検査（HBs抗原，HBs抗体，HBc抗体），HCV抗体，HIV-1/2抗体，ヒトT細胞白血病ウイルス1型（human T cell leukemia virus type 1: HTLV-1）抗体，ヒトパルボウイルスB19抗原検査を対象として，2007年までは凝集法により行っていた．凝集法は多数検体のスクリーニングに適している反面，客観的な数値結果が得られないことから，判定の基準として凝集写真を用い検体の希釈機や試薬の分注機を用いるなど，検査精度向上の改善が問題とされていた．この問題に対応するため検体・試薬の希釈分注と凝集パターン判定の一連の操作が機械化された自動検査機を1995年に導入し，その分析メソッドと精度管理方法を統一して検査精度の向上を図った．2008年からは化学発光酵素免疫測定法（chemiluminescence enzyme immunoassay: CLEIA）による自動検査機を導入し，検出感度や検査結果の客観性向上を図っている．

NAT導入当初は自動核酸抽出機の処理速度の問題から，血清学的検査陰性検体500本をプールしてPCR法（polymerase chain reaction）によりNATを行っていた．処理速度の向上した自動核酸抽出機の導入に伴い，翌2000年2月から50本プールに，さらに2004年8月からは20本プールへとプールサイズを縮小し，ウイルスの検出感度を向上させた．他方，献血受付時の本人確認，問診の強化，新鮮凍結血漿（fresh frozen plasma: FFP）の貯留保管（6カ月間）などとの相乗効果により輸血用血液の安全性は飛躍的に高められた．2008年8月には核酸抽出から増幅・検出までを自動で行う第二世代のNAT試薬・NAT機器へ変更となり，検査に使用する検体量も200 μLから850 μLへと4.25倍増量し検出感度の向上が図られた．2014年8月からは個別検体をTMA法（transcription mediated amplification）によりNATを行っている．

2 遡及調査

日本赤十字社は2004年に徹底した遡及調査の実施を国から求められた．遡及調査とは，「血液製剤等に係る遡及調査ガイドライン」（厚生労働省医薬食品局）によって「病原体の存在が疑われた供（献）血者の過去の供（献）血血液又は輸血等により感染が疑われた血液製剤等に関する情報及びこれらの供（献）血血液から製造された血液製剤の情報，当該製剤が投与された患者の感染に係る情報等を収集し，それを科学的に分析・評価することである」と定義されている．具体例を挙げると，過去の献血時の検査結果が「陰性」であった献血者が，次回の献血時に「陽性（陽転）」となった場合や，献血後に体調が悪くなって受診したところ，感染症と診断されて血液センターに連絡があった場合のように，献血者からの情報により輸血に使用された血液中に感染性病原体が含まれる可能性が判明する場合がある．過去の献血血液や体調悪化前の献血血液が，感染してから検査で陽性となるまでのウインドウ期に採血された可能性があるためである．また，輸血を受けた患者の感染症マーカーが陽転して感染が疑われた場合のように，医療機関（患者）からの情報により遡及調査が開始される場合もある．

いずれの場合も保管検体（1996年9月からす

表1 輸血による肝炎発生状況

年	HBV	HCV	HEV
2000	5	0	0
2001	7	0	0
2002	8	0	1
2003	13	0	0
2004	20	0	2
2005	11	1	1
2006	6	1	1
2007	13	1	0
2008	4	0	2
2009	7	0	1
2010	11	2	0
2011	13	0	0
2012	6	0	4
2013	7	1	1

べての献血血液の一部を冷凍保存し，非溶血性副作用や感染症症例の因果関係を確認するために使用している：11年間保存)や貯留保管中の凍結血漿を用いて高感度NATでウイルスの検出が行われる．ウイルスが陽性の場合はもちろん，陰性の場合も詳細な情報が医療機関に伝えられ，当該血液の輸血を受けた患者の観察を依頼している．輸血に使用された血液からウイルスが検出され，患者の感染症マーカーが陽転している場合には，双方のウイルスの相同性が検討され因果関係の有無を特定する．

3 輸血後肝炎発生状況

表1に2000年からの輸血後肝炎発生状況を示す．NAT開始前はHBVの感染が年間に約20例，HCV感染が5-7例認められていた．すべての輸血用血液にNATが導入された2000-02年は，HBV感染が年間5-8例でHCVの感染は2004年まで確認されなかった．2004年から徹底した遡及調査を実施したためHBV感染の例数が増加し，NAT導入以後確認されていなかったHCV感染も2005年から3年連続で1例ずつ確認された．このHCV感染の3例中2例は遡及調査によって判明した事例である．2010年には1人の献血者由来の赤血球製剤と新鮮凍結血漿で2人の患者への感染が確認された(採血は2006年)．2008年に感染症血清学的検査機器ならびにNATの試薬・機器も第二世代へと更新され，検出感度の向上が図られた．これらの変更により輸血用血液の安全性はさらに高まったと考えられたが，2009年以降もHBV輸血感染事例は年間7-13例が確認され，減少することはなかった．これはHBV DNA検出能が高まったことにより，輸血感染の解析感度も高くなったことで，今まで見逃されていた感染例が検出可能となったためと考えている．2013年にもHCV感染が1例確認されているが，この例も遡及調査により判明している．

E型肝炎ウイルス(hepatitis E vires: HEV)によるE型急性肝炎は，衛生環境が整備されていない熱帯・亜熱帯地域で主に飲料水を介した流行が散見され，我が国を含めた先進諸国では流行地域への渡航による輸入感染症と認識されていた．しかし近年，渡航歴のないE型肝炎症例が先進諸国から報告され，2001年に我が国で最初となる国内感染例が報告された[3]．ついで兵庫県内でシカ肉の喫食による4人の急性E型肝炎患者のHEVと，冷凍保存されていたシカ肉に含まれるHEVとが遺伝子学的に同一であることが証明された[4]．2002年には北海道で輸血による感染が確認され[5]，それ以降2013年までに計13例の輸血感染が確認されている．E型肝炎診断薬(IgA抗体)の保険収載は2011年10月になってからであるため，輸血感染もほとんどが遡及調査(血漿分画製剤の原料受入れ試験)により判明している．現在E型肝炎はブタ，イノシシ，シカなどをreservoirとする人畜共通感染症であることが明らかとなり，感染症法による四類感染症に分類され，診断後直ちに届け出ることが義務づけられている．

また，**表1**には含まれていないが2010年に輸血A型肝炎ウイルス(hepatitis A virus: HAV)感染が確認されている．この事例は献血者が献血後に熱発し医療機関を受診，急性A型肝炎と診断され，医療機関より血液センターに連絡が入り遡及調査の対象となった．献血者保管検体を検査したところHAV RNA陽性が確認された．

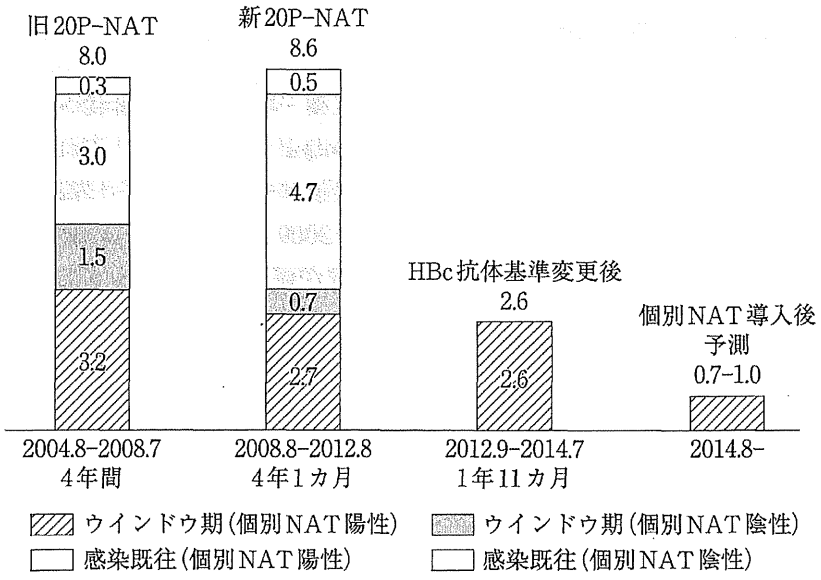

図2　年間あたりの輸血後HBV感染症例数

受血者は70歳代男性で腎疾患などによる入院患者であった．受血者の輸血前検査結果は，HAV RNA陰性，HAV-IgM抗体陰性，HAV-IgG抗体陽性であったが，輸血後5週目にHAV RNAが陽転化していた．供血者および受血者のHAV塩基配列は調査した2領域において完全に一致した．受血者は経過観察中に肝機能障害を認めず，輸血3カ月後にはHAV RNA陰性が確認された．献血者・受血者の状況が全く同様な報告が2004年にスイスから[6]，また小児への輸血HAV感染が2014年に米国から報告されている[7]．

4　肝炎ウイルス対策

1）B型肝炎ウイルス

2008年にHBs抗原検査が従来の凝集法（RPHA法）からCLEIA法に変更となり，HBs抗原検査の検出感度は100倍以上向上した．HBs抗原検査の検出感度向上に伴いHBV感染初期の検体を効率良く検出することが可能となった．また，同時期にNATの検出感度向上でHBV感染既往検体（HBs抗原陰性・HBV DNA陽性のHBVオカルト感染）からのHBV DNA検出能が著しく高まった．2007年までの輸血HBV感染例は，その半数以上がウインドウ期の血液による感染であったが，これらの変更により2010年のHBV感染例では原因となった献血者10人（感染事例は11例）のうち7人がHBc抗体陽性の感染既往者で，ウインドウ期の献血者は3人のみであった．日本赤十字社ではそれまでHBc抗体低力価陽性血液を輸血用血液として使用してきたが，前述のようにHBVオカルト感染血液での輸血感染が明らかになってきたことから，さらなる安全対策への検討を開始した．様々な検討の結果，2012年8月よりそれまで輸血用血液として使用していたHBc抗体低力価陽性血液の使用を止め，HBc抗体陰性の血液のみを供給するようになった（HBs抗体200 mIU/mL以上を除く）．

2008年以前の旧20本プールNATと2008年以降の新20本プールNATを比較すると，年間あたりの感染事例数はほぼ変わらないが，新20本プールNAT下では半数以上が感染既往血液による感染であった．HBc抗体陽性血液の使用を止めてからは感染既往血液による感染がなくなり，HBV感染初期の20本プールNAT陰性・個別NAT陽性の血液が原因となっている（**図2**）．

正常ヒト肝細胞をもつキメラマウスへのHBV感染実験では，10コピーのHBVで感染が成立し[8]，チンパンジーへの感染実験でも10コピーのHBVで50％が感染すると報告されている[9]．

このように非常に少量のHBVでも感染することが知られており，輸血HBV感染事例でも個別NAT陰性血液による感染が数例確認されている．これらの事例では当該献血者の次回献血血液中のウイルスや，当該血液中のHBV DNAを濃縮するなどして解析を行い，受血者の発症時ウイルスとの遺伝子相同性が確認されている．このような血液による感染は現在の最新技術を駆使しても防ぐことは困難であり，感染機会のあった献血者をどのようにして排除するかが問題となっている．これらの事実から，個別NAT導入以降も年間あたり0.7–1.0例の輸血HBV感染事例が発生する可能性が残存している(**図2**)．

2）C型肝炎ウイルス

HCV感染では，感染から末梢血中にHCV RNAが検出される濃度に上昇するまでの期間(pre-ramp up phase)が1–2カ月あるといわれ[10]，その後ウイルス濃度は急激に上昇して数日で高濃度水平状態に達する．pre-ramp up phaseで採血された血液はほとんど感染性がないことがチンパンジーの感染実験で示されており[11]，HCV抗体検出前のウインドウ期血液は個別NATによってほぼ排除できると考えられている．2000年に個別NAT陰性血液による輸血感染例が報告されているが[12]，当時のHCV-NATの感度は約100コピー/mLで，現在は約10倍程度検出感度が向上している．したがって，個別NAT導入以降輸血HCV感染は極めてまれとなると予測されているが，チンパンジーへのHCV感染実験では20コピーのHCVで感染すると報告されており[13]，輸血感染のリスクが全くなくなったわけではない．

3）E型肝炎ウイルス

2002年に輸血によるHEV感染例が初めて報告[5]されたことを受けて，2003年4月から2004年3月までの1年間に，ALTが高値(200IU/L以上)となった全国の献血者血液について，HEV RNA，HEV-IgG抗体，HEV-IgM抗体の調査を実施した[14]．その結果，HEV RNAおよびHEV-IgM抗体は東日本が高率で，HEV-IgG抗体は北海道，関東甲信越，九州で全国平均を上回る結果となった．特に北海道ではすべてのマーカーが非常に高率であったこと，肝炎が重症化しやすいといわれている遺伝子型4が多かったことなどから，研究的な調査として北海道内の全献血者血液のHEV RNA検査と，献血時の問診で生肉や生レバーの喫食状況の聴取を開始した．北海道での研究的20本プールHEV-NATでは，2005–14年の264万献血中289例(0.011％，男性218，女性71)から陽性が確認され，このうち献血前の内臓肉喫食率は70％と高率でHEV感染との関連を裏づける結果となった．北海道内での研究調査は現在も継続しており，HBV，HCV，HIVのNATと同様に2014年8月からは個別検体によるNATを行っている．この研究的HEV-NATにより2006年3月以降は北海道内での輸血HEV感染は確認されていない．

東京都内でも2006年に調査を行い，44,000献血から3例(0.0068％)のHEV RNA陽性が確認されている．HEVによる肝炎は時に中程度の肝障害を惹起するが，ほとんどの場合は無症状・無所見である．そのため確認される輸血HEV感染は**表1**のように年間約1例であるが，実際にはどれくらいの輸血感染が発生しているのかは不明である．また，北海道以外の地域からも重症化しやすいといわれている遺伝子型4検出の報告がなされるようになり[15]，HEV-NATの全国導入の是非が費用対効果を含めて議論されている．

最近，厚生労働省はブタの生レバーや生肉の飲食店などでの提供を禁止するとした食品衛生法の新たな規格基準を公表した．この基準が厳格に準拠されれば，国内のHEV感染は大幅に減少すると考えられ，それに伴い輸血感染のリスクも低下すると思われる．

おわりに

2015年5月末日までで個別NAT導入後10カ月が経過したが，導入後に採血された血液によるHBV，HCVおよびHIV感染は1例も確認されていない．しかしながら，輸血療法では大量の血漿が受血者に輸注されるため，スクリーニング感度がいくら向上しても輸血感染のリスクはゼロにはならない．1mL中に1コピーのウイ