cases.[41] In our study, analyses with adjustment for lifestyle-related factors and radiation dose in non-B, non-C subjects showed that the risk of non-B, non-C HCC is significantly higher in the middle or highest tertile of serum CRP levels than in the lowest tertile, and that the risk increases with elevated serum levels of CRP (though only with marginal statistical significance). This result is consistent with published findings that background liver disease of non-B, non-C HCC may be partially caused by NASH or steatohepatitis.[40,41]

Several studies have reported that higher serum IL-6 level precedes the development of HCC in female chronic hepatitis C patients or chronic hepatitis B patients.[20,21] Estrogen-mediated inhibition of IL-6 production by Kupffer cells may explain such gender disparity in HCC development.[22,42–44] An animal study also showed gender-based differences in IL-6 production associated with liver cancers.[22] Previous studies have also demonstrated that serum IL-6 level increases in patients with established HCC.[45] IL-6 is a multifunctional cytokine that plays a prominent role in immune response, cell survival, apoptosis and proliferation.[46] IL-6 produced by inflammatory and stromal cells within the tumor microenvironment binds to gp80 (IL-6 receptor)/gp130 complex, leading to constitutive Janus kinase (JAK) activation and STAT3 phosphorylation, which regulates oncogenic gene expression mediating proliferation and preventing apoptosis.[24] Early studies reported that IL-6 and STAT3 are involved as protumorigenic agents in many cancers, including those of the colon, lung, breast, prostate and ovary, as well as hematological cancers.[46] In our study, the association between serum levels of IL-6 and HCC risk was significant after adjusting for HBV and HCV infection, lifestyle-related factors and radiation dose. Elevated serum levels of IL-6 were associated with increased risk of HCC irrespective of gender. Additionally, analyses with adjustment for lifestyle-related factors and radiation dose in HCC cases and controls of non-B, non-C type showed that non-B, non-C HCC risk is significantly higher in the middle or highest tertile of serum IL-6 levels than in the lowest tertile, and that the risk significantly increases with elevated serum levels of IL-6. These results are consistent with published findings that elevated IL-6 level is associated with the development of type 2 diabetes or insulin resistance,[47] which are considered to be factors contributing to progression in non-B, non-C HCC as well as HCC.

Obesity and diabetes mellitus have recently earned recognition as risk factors for HCC.[4–9] Our previous study[3] also demonstrated that obesity 10 years before HCC diagnosis was an independent risk factor for HCC, and that there was a significant multiplicative interaction in HCC risk between obesity and HCV infection. Obesity contributes to a high rate of visceral fat storage. Increases in production of cytokines such as TNF-α, IL-6, monocyte chemoattractant protein-1 and leptin secreted from adipose tissue and/or macrophages accumulated in such tissues cause hepatic steatosis and oxidative stress through insulin resistance, resulting in the development of HCC. A recent experimental study using a mouse model indicated that obesity promotes HCC development by enhancing production of the tumor-promoting cytokines such as IL-6 and TNF, which cause hepatic inflammation and activation of the oncogenic transcription factor STAT3.[23] In our study, elevated serum levels of IL-6 were significantly associated with increased risk of HCC, especially among subjects with obesity, after adjusting for all other categories of the other risk factor. That trend changed little when the association between IL-6 levels and non-B, non-C HCC risk was examined. Other factors related to HCC risk among obese subjects such as genotype may affect the interaction between IL-6 and obesity, when taking into account the fact that correlations between serum levels of IL-6 and BMI were not significant among HCC cases and controls. Nevertheless, monitoring of IL-6 levels may be crucial to early detection of HCC irrespective of HBV and/or HCV infection, especially for individuals with chronic liver disease or fatty liver disease with obesity.

The strengths of our study include its prospective cohort base with high follow-up rate and nested case–control design, which minimize selection bias. It is difficult and expensive to perform full cohort analyses of serum biomarkers such as IL-6 and CRP, whereas the nested case–control design used here can provide substantial reductions in cost and effort with little loss of statistical efficiency.[48] We also incorporated, in a strict and in-depth manner, hepatitis virus infection status of HCC cases measured before diagnosis (measured at comparable ages among matched controls). Furthermore, we included such potential HCC risk factors as alcohol consumption, smoking habit and BMI in the multivariate analyses, because several studies have demonstrated that inflammatory markers including CRP and IL-6 levels are associated with such lifestyle-related factors.[16,17] However, we cannot completely exclude the possibility of residual confounding.

A limitation of our study is that use of hormones, aspirin and nonsteroidal anti-inflammatory drugs, which are related to CRP levels, could not be adjusted as confounders, because participants have only been asked detailed information on such kinds of medication since 1991. Another is that we used stored sera obtained within 6 years before HCC diagnosis. The reason is that to render primary diagnosis of HBV and/or HCV infection status of cases and controls of serum samples obtained from study participants between 1970 and 2002, *de novo* HCV infection in particular could not be denied outright regarding those obtained between 1970 and 1989. Therefore, the findings of elevated IL-6 levels associated with HCC risk (also measured within 6 years of diagnosis) may include a mixture of precancerous change and defense against tumor formation or growth. It suggests that elevated IL-6 levels may represent not cause but effect for increased risk of HCC, although causality cannot be inferred from our study. However, for early identification and management of HCC, measurement and monitoring of IL-6 levels for individuals with chronic liver disease or fatty liver disease may be meaningful, irrespective of HBV and/or HCV infection.

In conclusion, elevated serum levels of IL-6 were associated with increased risk of HCC, even after adjusting for HBV or HCV infection, alcohol consumption, smoking habit, BMI and radiation dose. Elevated IL-6 levels associated with non-B, non-C HCC risk were also observed, although it was estimated among a relatively small number of non-B, non-C HCC cases. Moreover, elevated serum levels of IL-6 were significantly associated with increased risk of HCC, especially among subjects with obesity. Elevated serum levels of CRP were only marginally associated with increased risk of non-B, non-C HCC, whereas monitoring of CRP and IL-6 levels in combination with tumor markers may be more robust in predicting subsequent HCC among individuals with non-B, non-C liver disease. An in-depth understanding of the mechanisms by which IL-6 levels are associated with increased risk of HCC, independently of hepatitis virus infection, lifestyle-related factors and radiation exposure, should lead to better prevention and therapeutic strategies.

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Original Article

Percutaneous transvenous embolization for portosystemic shunts associated with encephalopathy: Long-term outcomes in 14 patients

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Aim: To evaluate the clinical outcomes of percutaneous transvenous embolization (PTE) for portosystemic shunt (PSS) associated with encephalopathy

Methods: Fourteen patients with portosystemic encephalopathy (PSE) were enrolled in this retrospective cohort study. We evaluated technical success, clinical success, complication and outcomes.

Results: In cases in which PSS was one of main causes of PSE, three also had splenorenal shunts, four gastrorenal shunts, four superior mesenteric vein systemic shunts, one inferior mesenteric vein systemic shunt and two main trunk of portal vein inferior vena cava shunts. We used only ethanolamine oleate (EO) in five; EO and coils in five; EO, coils and n-butyl 2-cyanoacrylate (NBCA) in two; and coils and NBCA in two patients as embolic materials. The rate of primary and secondary technical success was 93% (13/14 patients) and 100%, respectively. No major complications were encountered related to PTE. Follow-up period was a median of 27 months (range, 12–79). All patients had sustained disappearance of PSE. PSE recurred in one patient because of another PSS development. Thus, clinical success was achieved in 93% (13/14 patients). The ammonia levels 1 year after PTE were significantly improved compared with pre-PTE (median, 102 vs 41 μmol/L) and maintained lower levels 2 and 3 years later. Child–Pugh scores did not change significantly. Esophageal varices were aggravated in 29% (4/14 patients). Five patients died, but no death of hepatic failure related to PTE was encountered.

Conclusion: PTE could be one of the useful treatment options for PSE.

Key words: balloon-occluded retrograde transvenous obliteration, encephalopathy, percutaneous transvenous embolization, portal systemic shunt

INTRODUCTION

THERE ARE TWO types of hepatic encephalopathy: portosystemic encephalopathy (PSE) and end-stage hepatic encephalopathy in decompensated cirrhosis.[1,2] PSE is caused by portosystemic shunts (PSS), including splenorenal (SR) shunts, gastrorenal (GR) shunts, superior mesenteric vein (SMV) systemic shunts, inferior mesenteric vein (IMV) systemic shunts and portal vein (PV) inferior vena cava (IVC) shunts. PSE can be treated with surgery or interventional radiology,[3–8] or medication.[9,10] Surgical treatment is ligation of shunts.[11,12] However, surgery is an invasive strategy and may be limited due to severe liver dysfunctions. Medical treatment is to eliminate ammonia from the gastrointestinal tract, for example, osmotic diarrhea, reduction of dietary protein intake and administration of lactulose to reduce serum ammonia levels. Neomycin also may be given to suppress bacterial flora preventing them from converting amino acids into ammonia. Neomycin is admin-

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istrated p.o. or by retention enema.[9,10] But these medical managements have not been always sufficient, especially in PSE.

Balloon-occluded retrograde transvenous obliteration (B-RTO) has been used for the treatment of solitary gastric varices,[13–17] and it has been reported as a useful therapy for PSE recently,[18–21] mainly for SR and GR shunts, but rarely for SMV and IMV systemic shunts among others. Ethanolamine oleate (EO) has been used in B-RTO for an embolic material as a sclerosing agent. Also, coils[22,23] and n-butyl 2-cyanoacrylate (NBCA)[24,25] have been used in embolization of PSS.

We defined occlusions of PSS including B-RTO using a catheter by percutaneous transvenous embolization (PTE). PTE has been safely performed for PSE with almost complete eradication. At present, however, the majority of reports have been case reports or short-term results.[4–8] The long-term outcome of more than 1 year after PTE has not yet been fully demonstrated.

In the present study, we described the technical and clinical success rate, the complications, and the outcome in long-term periods of more than 1 year.

METHODS

THIS STUDY WAS conducted according to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients or their families before treatment and at the time of enrollment.

Patient

Fourteen consecutive patients with PSE treated with PTE at our institution between April 2006 and October 2011 were enrolled in this retrospective cohort study. All of these patients were poorly responsive to medical therapy for PSE.

Patient characteristics are shown in Table 1. The study included nine men and five women with a median age of 71 years (range, 55–74). All patients had liver cirrhosis, the causes were viral liver cirrhosis in 10 patients (nine patients were positive for anti-hepatitis C virus antibody and one patient was positive for hepatitis B surface antigen), and alcoholic liver cirrhosis in four patients. Child–Pugh scores[26] were 6 in one, 7 in five, 8 in four, 9 in one and 10 in three patients. All patients had dominant encephalopathy and the grade of encephalopathy by criteria based on the West Haven Criteria[27] were grade 1 in five, 2 in six, 3 in two and 4 in one patient. The median serum ammonia level was 104 μmol/L (range, 79–175). Other blood test results are shown in Table 1.

Table 1 Patient characteristics

Parameters	$n = 14$
Age (years)†	71 (55–74)
Sex (male/female)	9/5
Etiology (HBV/HCV/alcohol)	1/9/4
Child–Pugh score (6/7/8/9/10)	1/5/4/1/3
Grade of hepatic encephalopathy (1/2/3/4)†	5/6/2/1
Ammonia (μmol/L, range)‡	104 (79–175)
Total bilirubin (mg/dL, range)‡	34 (19–92)
Aspartate aminotransferase (IU/L, range)‡	35 (18–81)
Alanine aminotransferase (IU/L, range)‡	22 (13–67)
Alkaline phosphatase (IU/L, range)‡	346 (190–713)
γ-Glutamyltransferase (IU/L, range)‡	20 (7–145)
Albumin (g/dL, range)‡	3.3 (2.6–3.7)
Creatinine (mg/dL, range)‡	0.84 (0.36–1.23)
Platelet count ($\times 10^4$/μL, range)‡	8.4 (3.5–29.7)
Ascites (present/absent)	6/8
HCC (present/absent)	9/5
HCC stage (I/II/III/IVa/IVb)	2/3/3/0/1

†Grade is by West Haven Criteria.
‡Data are mean values (range).
HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

Nine patients had complicating hepatocellular carcinoma (HCC). HCC staging by the Liver Cancer Study Group of Japan[28] were I in two, II in three, III in three and IVb in one patient.

PTE

Selective angiography from the celiac and superior mesenteric arteries, and computed tomography during arterial portography via superior mesenteric or/and splenic arteries using an angio-CT system (Miyabi [Siemens, Erlangen, Germany] or INFX-8000C+ Aquilion [Toshiba, Otawara, Japan]) were performed before PTE in order to evaluate portosystemic collaterals via unilateral femoral artery.

We inserted a 5-Fr catheter with a 1 or 2-cm diameter balloon (Selecon balloon catheter; Terumo Clinical Supply, Gifu, Japan) into the draining vein of the PSS through an IVC via a right femoral or right jugular vein under local anesthesia. PTE was commonly performed using 5% EO (Oldamin; Takeda Pharmaceutical, Osaka, Japan) mixed with iopamidol (EOI) (Iopamiron 300; Bayer Health Care, Osaka, Japan) under balloon occlusion, so-called B-RTO (patient no. 1–8, 10–12 and 14) (Fig. 1, Table 2). If necessary, minor collateral vessels of the PSS were embolized by 50% glucose solution and microcoils before EOI injection. The amount of EOI was

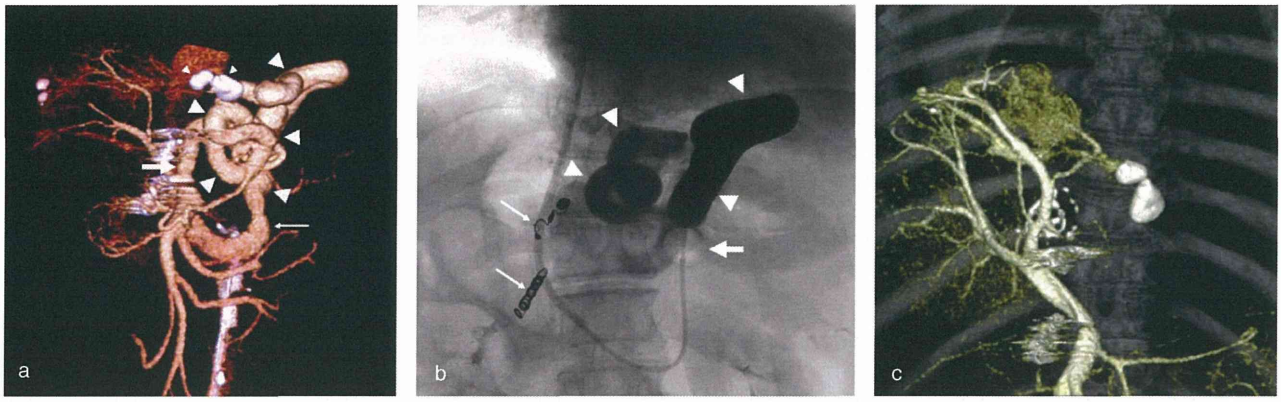

Figure 1 Percutaneous transvenous embolization (PTE) with 5% ethanolamine oleate iopamidol (EOI) for gastrorenal (GR) shunts (patient no. 5). (a) 3-D image obtained by computed tomographic arterial portography via a superior mesenteric artery shows GR shunt (large arrowheads) from a right gastric vein (large arrow) to a left renal vein (small arrow). Small arrowheads indicate the calcified lymph nodes' swelling around a stomach. (b) Fluoroscopic spot image shows a total of 15 mL (0.29 mL/kg bodyweight) of 5% EOI injected from a 5-Fr balloon catheter (large arrow) via a right jugular vein into the GR shunt (arrowheads). Small arrows indicate microcoils at a right gastric and gastroduodenal arteries for hepatic arterial infusion chemotherapy using an implanted hepatic arterial port system for hepatocellular carcinoma treatment, which was removed during PTE. (c) 3-D image obtained by intravenous contrast-enhanced CT 27 months after PTE shows no appearance of the GR shunt.

defined as below 0.4 mL/kg bodyweight in one session to reduce the side-effects. In some cases, we combined some types of microcoils (Tornado Coil [Cook, Bloomington, IN, USA], Micronester Coil [Cook], Interlocking Detachable Coil [Boston Scientific/Target, Fremont, CA, USA], Guglielmi Detachable Coil [Boston Scientific, Cork, Ireland], Interlock Coil [Boston Scientific] and C-stopper Coil [Piolax, Yokohama, Japan]) and/or

Table 2 Results of PSS and PTE

Patient no.	PSS	Diameter of PSS (mm)†	Embolic materials			Technical success		Clinical success
			Amount of 5% EOI (mL)	No. of coils	Amount of NBCA (mL)	Primary	Secondary	
1	SR	21	20	–	–	Complete	–	Achieved
2	SR	12	12.5	3	–	Complete	–	Achieved
3	SR	21	20	3	–	Partial	Complete	Achieved
4	GR	19	20	–	–	Complete	–	Achieved
5	GR	10	15	–	–	Complete	–	Achieved
6	GR	20	31	4	–	Complete	–	Achieved
7	GR	16	17	–	–	Complete	–	Achieved
8	SMV-IVC	10	20	5	–	Complete	–	Achieved
9	SMV-IVC	15	–	14	5	Complete	–	Achieved
10	SMV-IVC	23	20	12	5.5	Complete	–	Achieved
11	SMV-IVC	8	4.5	2	0.2	Complete	–	Achieved
12	IMV-IVC	15	5	6	–	Complete	–	Achieved
13	MPV-IVC	11	–	9	0.5	Complete	–	Recurrence
14	MPV-IVC	24	15	–	–	Complete	–	Achieved

†Mean.
EOI, 5% ethanolamine oleate with iopamidol; GR, gastrorenal; IMV, inferior mesenteric vein; IVC, inferior vena cava; MPV, main trunk of portal vein; NBCA, n-butyl 2-cyanoacrylate; PSE, percutaneous transvenous encephalopathy; PSS, portal systemic shunt; PTE, percutaneous transvenous embolization; SMV, superior mesenteric vein; SR, splenorenal.

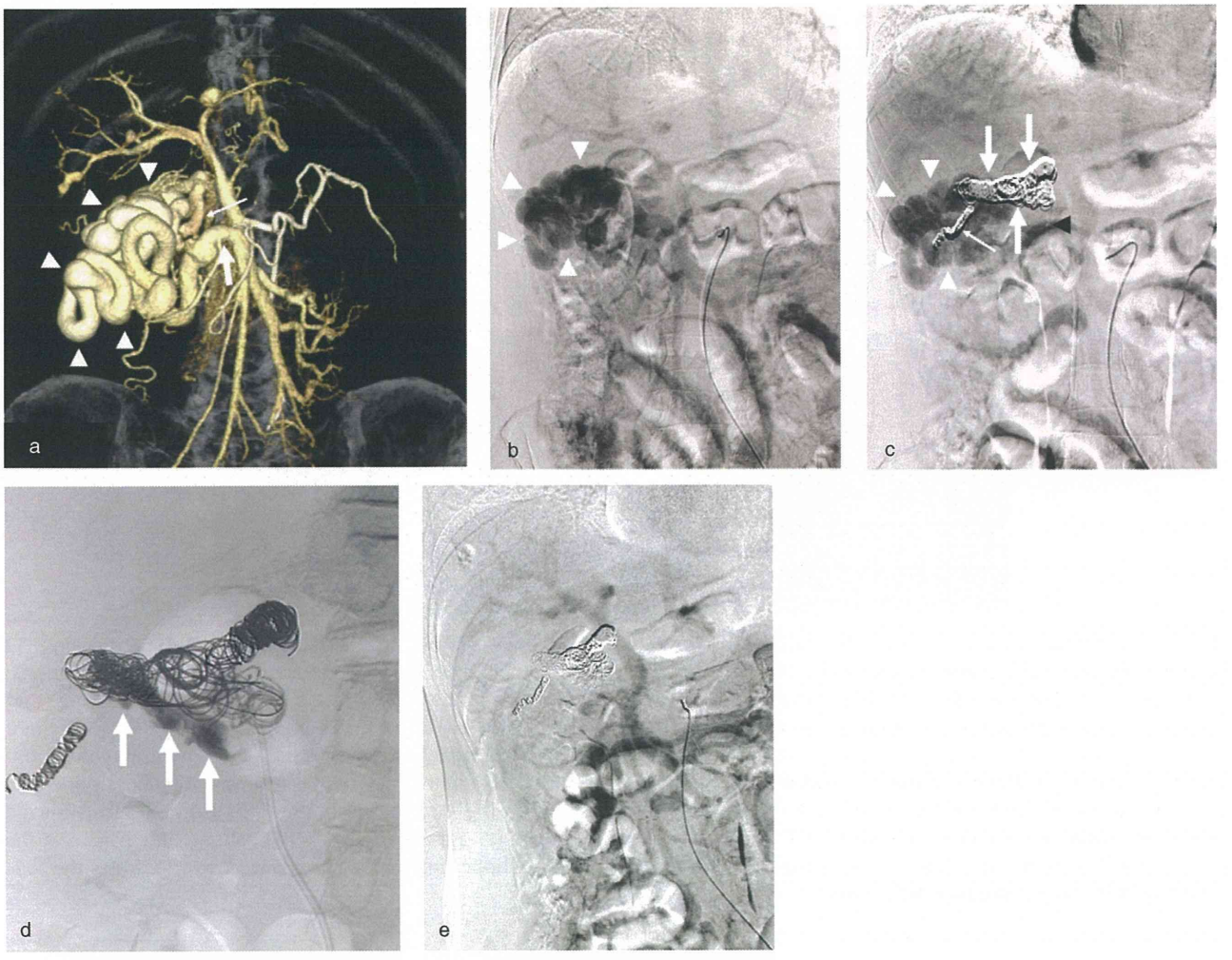

Figure 2 Percutaneous transvenous embolization (PTE) with coils and n-butyl 2-cyanoacrylate (NBCA) for a large superior mesenteric vein (SMV) inferior vena cava (IVC) shunt (patient no. 9). (a) 3-D image obtained by computed tomographic arterial portography via a superior mesenteric artery shows a large SMV-IVC (arrowheads) shunt. Large and small arrows indicate the inflow from an SMV and outflow to an IVC, respectively. (b) A superior mesenteric angiography shows a large SMV-IVC shunt similar to (a) (arrowheads). (c) A superior mesenteric angiography still shows an appearance of SMV-IVC shunt (white arrowheads) after placements of 25 microcoils at the main outflow vein to the IVC (large arrows) and 11 microcoils at the collateral vein (small arrow) via a right femoral vein using a 3-Fr microcatheter through a 5-Fr balloon catheter (black arrowhead). (d) NBCA–lipiodol mixture (5 mL; ratio 1:1) (arrows) was injected from a microcatheter under balloon occlusion to fill the gaps between the coils for a more complete blockage of the shunt. (e) A superior mesenteric angiography shows no appearance of the shunt after coils and NBCA embolization.

NBCA (Histoacryl; Aesculap, Tuttlingen, Germany)–lipiodol (André Guerbet, Aulnay-sous-Bois, France) mixture with EOI in order to reduce the amount of EOI using a microcatheter. We left the balloon catheter in the draining vein with the balloon inflated overnight and removed it after retrograde venography from the balloon catheter revealed complete obliteration. If obliteration of PSS was insufficient on retrograde venography, additional PTE was subsequently performed until disappearance of inflow vessels. To prevent renal dysfunction related to hemolysis occurring as a side-effect of EOI, 2000–4000 units of haptoglobin were administrated to all patients before PTE.

When the PSS was large (Fig. 2) or the connection between the shunt and portal vein was too short (Fig. 3), PTE was performed with NBCA and 0.035-inch

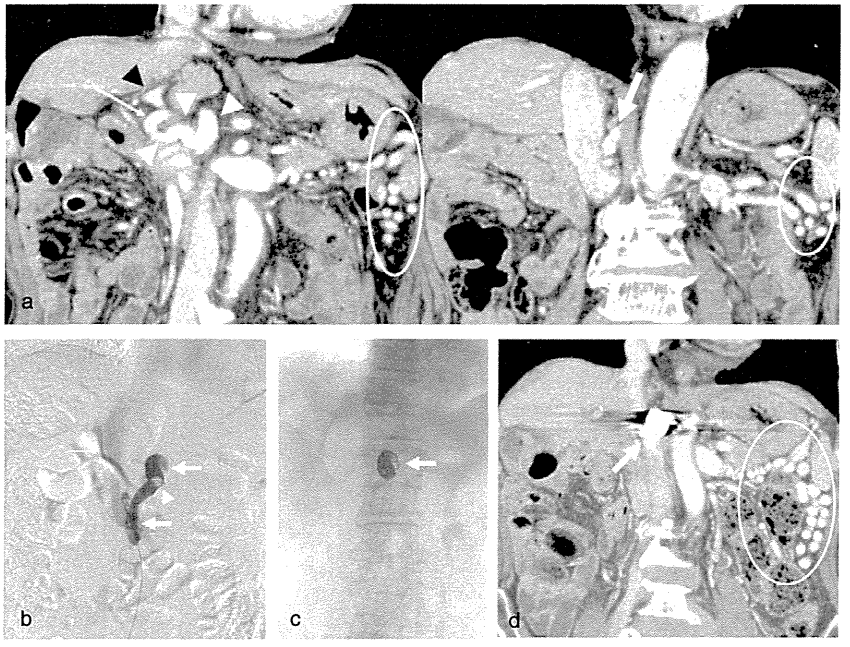

Figure 3 Percutaneous transvenous embolization (PTE) with coils and n-butyl 2-cyanoacrylate (NBCA) for a short portal vein (PV) inferior vena cava (IVC) shunt (patient no. 13). (a) Coronal reconstructed images of intravenous contrast-enhanced computed tomography (CT) shows a short PV-IVC shunt (white arrowheads). Right, ventral slice image; left, dorsal slice image. Large and small arrows indicate the inflow from IVC to the outflow to PV (black arrowhead). Note another PSS of splenorenal (SR) shunt (elliptic circles). (b) Retrograde venography from a 5-Fr balloon catheter via a right femoral vein shows the outflow of the shunt (large arrows) into IVC (small arrow). Arrowhead indicates the tip of a balloon catheter. (c) Outflow vein was embolized alone with nine microcoils and 0.5 mL NBCA–lipiodol mixtures, ratio 1:2 (arrow), using a coaxial technique. (d) Coronal reconstructed images of intravenous contrast-enhanced CT 7 months after PTE shows no appearance of PV-IVC shunt. But it reveals the development of SR shunt (elliptic circle) compared with that before PTE (elliptic circles in [a]) together. Arrow indicates the coils and NBCA in PV-IVC shunt embolization.

coils (MReye Embolization Coil; William Cook Europe, Bjaeverskov, Denmark) or microcoils to occlude the PSS at the short portion.

In the above procedures, we used a 3-Fr microcatheter through the balloon catheter co-axillary in case of necessity. Diameters of coils ranged 4–30 mm. The ratio of NBCA–lipiodol mixtures varied 1:1, 1:2 or 1:5.

Technical success

The therapeutic effects were evaluated by intravenous contrast-enhanced CT approximately 1 week after the treatment. When the contrast-enhanced CT scan showed PSS with low attenuation, partial enhancement and whole enhancement, we considered the obliterations to be complete, partial and failure, respectively.

Primary technical success was defined to be complete obliteration of PSS in CT after the first session. Secondary technical success was defined to be complete obliteration after the second session.

Clinical success

Clinical success was evaluated by grade of coma. Disappearance of encephalopathy for more than 1 week was defined as clinical success. If not, it was defined as failure. After achievement of clinical success, relapse of encephalopathy was defined as recurrence.

Complications

We adequately assessed complications related to the procedures according to the Society of Interventional Radiology[29] for PTE. Minor complications did not to require medical attention. Major complications required therapy, or resulted in permanent adverse sequelae and death.

Outcomes

The serum ammonia level and hepatic functional reserves based on results of blood tests of serum total

bilirubin level, albumin and prothrombin time percentage activity were estimated consecutively 1 day, 3 months, and 1, 2 and 3 years after PTE. The changes of Child–Pugh scores were calculated at 3 months, and 1, 2 and 3 years later. Diagnostic imaging by CT was performed simultaneously. Endoscopy also was performed after PTE in the follow-up periods. Also, the overall cumulative survival rate and the cause of death were reviewed.

Statistical analysis

Changes of serum ammonia levels and serum laboratory values of total bilirubin, albumin and prothrombin time percentage activity were assessed by repeated measures ANOVA. Analysis was performed using the Mann–Whitney *U*-test. $P < 0.05$ was considered significant. All analyses were performed with SPSS software ver. 11.

RESULTS

PSS

PORTOSYSTEMIC SHUNTS AS the main cause of PSE included SR shunts in three, GR shunts in four, SMV systemic shunts in four, IMV systemic shunt in one and PV-IVC shunts in two patients. One patient had both SR and SMV systemic shunts, the other patient had both SR and PV-IVC shunts. The median maximum diameter of PSS was 15.5 mm (range, 8–24) (Table 2).

PTE procedures

Percutaneous transvenous embolization with 5% EOI alone (patient nos. 1, 4, 5, 7 and 14) (Fig. 1), EOI and coils (patient nos. 2, 3, 6, 8 and 12), and EOI, coils and NBCA (patient nos. 10 and 11) were performed in five, five and two patients, respectively. The median amount of 5% EOI was 18.5 mL (range; 4.5–31) and median dose of EOI for bodyweight was 0.23 mL/kg (range, 0.07–0.4) in these patients.

Percutaneous transvenous embolization with coils and NBCA was performed for large (patient no. 9) (Fig. 2) and short shunts (patient no. 13) (Fig. 3) (Table 2).

Technical success

Primary technical success was achieved in 93% (13/14 patients). One patient (patient no. 3) achieved a partial success after the first session. This case obtained occlusion by re-PTE 13 months after the first PTE. Thus, secondary technical success was 100% (14/14 patients).

Clinical success

Portosystemic encephalopathy reversed rapidly in all patients on the day following PTE. One patient (patient no. 13) had recurrence 2 weeks after PTE. Thus, clinical success was achieved in 93% (13/14 patients). This recurrent case had both PV-IVC and SR shunts, but we achieved occlusion with PV-IVC shunt alone in this patient (Fig. 3).

Complications

Complications are shown in Table 3. There were no major complications and all complications were minor: pain in one (patient no. 4), fever of over 38°C in 10

Table 3 Complications

	Variety of embolic materials				
	EOI ($n = 5$)	EOI and coils ($n = 5$)	EOI, coils and NBCA ($n = 2$)	Coils and NBCA ($n = 2$)	Total
Major complications	–	–	–	–	None
Minor complications					
Pain	1	–	–	–	1
Fever elevation†	4	3	2	1	10
Gross hematuria	3	1	1	–	5
Increased transaminase‡	–	1	–	–	1
Jaundice§	1	3	–	–	4
Renal dysfunction¶	–	–	1	–	1

†More than 38°C.
‡More than threefold.
§Increased total serum bilirubin >2.0 mg/dL.
¶Increased serum creatinine >1.5 mg/dL.
EOI, 5% ethanolamine oleate with iopamidol; NBCA, n-butyl 2-cyanoacrylate.

(patient nos. 1, 3, 4, 6, 8, 9–12 and 14), gross hematuria in five (patient no. 1, 3, 4, 6 and 8), increased transaminase (less than three times the upper limit of normal) in one (patient no. 8), jaundice (total serum bilirubin, 2.0–3.0 mg/dL) in four (patient nos. 4, 6, 8 and 12) and renal dysfunction (serum creatinine, 1.5–2.0 mg/dL) in one patient (patient no. 5).

Outcomes

No one was lost to follow up. The median follow-up period in all patients was 27 months (range, 12–79).

Figure 4 shows the transitional change of serum ammonia, prothrombin time activity percentage, albumin and total bilirubin. With respect to changes in regular laboratory data, the ammonia levels 1 year after the PTE (median, 41 μmol/L; range, 13–98 μmol/L) were significantly improved compared with the baseline (median, 102 μmol/L; range, 79–175 μmol/L) ($P < 0.001$). Furthermore, the ammonia levels were maintained at low levels 2 and 3 years later. On the other hand, there were no changes in the level of prothrombin time activity percentage (60% [55–76%] vs 77% (39–112%), $P = 0.128$), albumin level (3.3 [2.6–3.7] vs 3.3 [2.6–4.0] g/dL, $P = 0.927$) and total bilirubin (1.1 [0.4–4.0] vs 1.0 [0.4–1.8] mg/dL, $P = 0.282$). Child–Pugh scores were also significantly unchanged (8 [6–10] vs 7 [5–11], $P = 0.104$).

Re-canalization of PSS did not appear in 14 patients on follow-up CT after achievement of technical success. However, an SR shunt was further implemented after

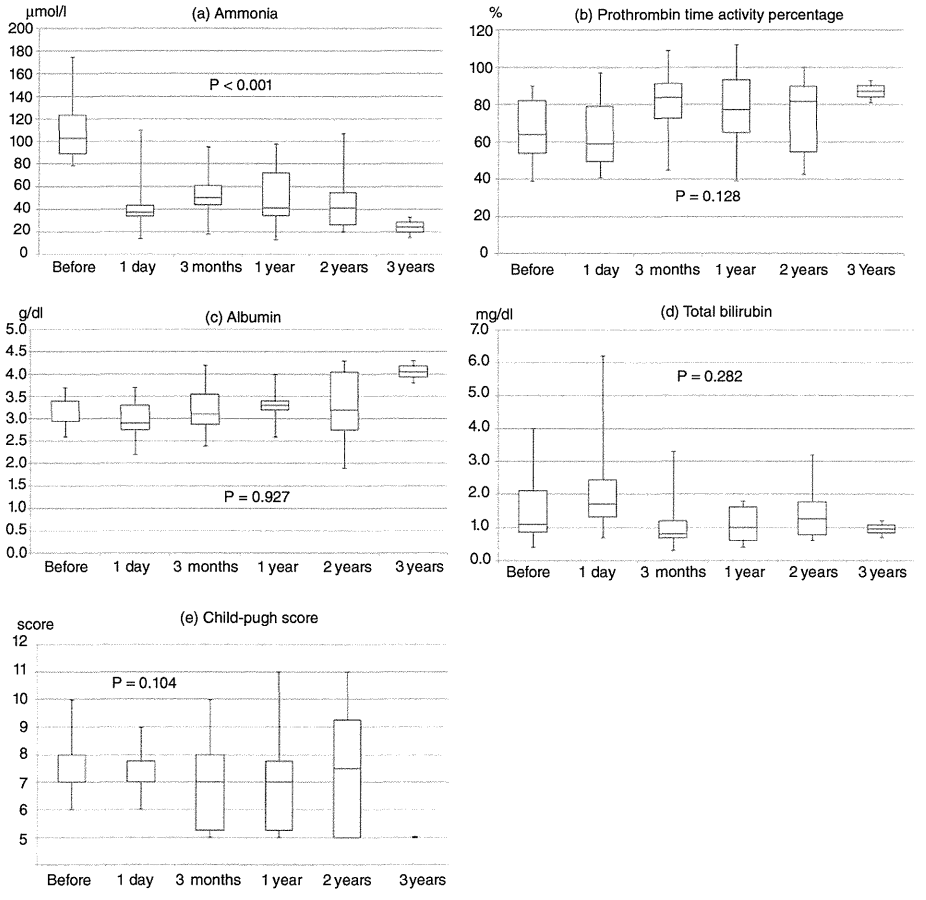

Figure 4 Transitional changes of (a) serum ammonia, (b) prothrombin time activity percentage, (c) albumin, (d) total bilirubin and (e) Child–Pugh score before and after treatment of percutaneous transvenous embolization (PTE). (a) Ammonia level was significantly improved (102 [79–175] vs 41 [13–98] μmol/L; $P < 0.001$) 1 year after the PTE compared with the baseline. (b–e) Prothrombin time percentage activity, albumin level, total bilirubin level and Child–Pugh score were not changed significantly between those before and 1 year after the PTE.

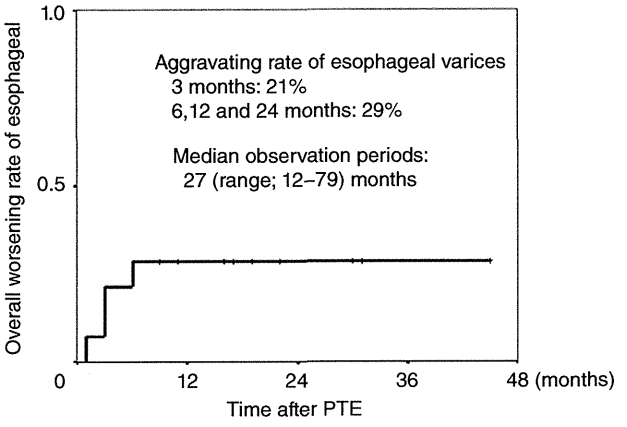

Figure 5 Kaplan–Meier analyses of cumulative aggravated esophageal varices.

the obliteration of PV-IVC shunt alone in one patient (patient no. 13).

Four patients (patient nos. 1, 5, 7 and 8) developed aggravated esophageal varices between 1 and 14 months after PTE. Figure 5 shows the rate of aggravated esophageal varices using the Kaplan–Meier method. Esophageal varices were found to have become aggravated from F1 to F2 or F3 and from RC0 to RC1 or RC2 or RC3.[30] The rate of bleeding was 14% (2/14 patients). The overall rate of aggravated esophageal varices was 21.4%, 28.6%, 28.6% and 28.6% at 3, 6, 12 and 24 months after PTE, respectively. These cases were resolved by endoscopic injection sclerotherapy or endoscopic variceal ligation. No patient had a hemorrhage from gastric varices in the follow-up periods.

Five patients died in the follow-up periods. The causes of death were HCC in two (patient nos. 4, 2 at 17 and 30 months after PTE, respectively), hepatic failure in one (patient no. 3 at 30 months after PTE), lung cancer in one (patient no. 1 at 76 months after PTE) and pulmonary hypertension in one (patient no. 6 at 31 months after PTE). The overall cumulative survival rate was 100%, 90% and 45% at 12, 24 and 36 months after PTE, respectively (Fig. 6).

DISCUSSION

IN THE PRESENT study, we analyzed the outcome and complications of 14 patients with PSE after PTE in long-term follow-up periods of more than 1 year. Primary and secondary technical success rates were 93% (13/14 patients) and 100% (14/14 patients). No major complications related to PTE procedures were encountered in any of the patients, but aggravated esophageal varices were developed in four patients during follow up. Clinical success rates were 93% (13/14 patients). The cause of failure in one patient was development of another PSS. Ammonia level had been significantly improved after PTE. Child–Pugh scores as hepatic functional reserves had not changed significantly.

We used 5% EOI, coil and NBCA as embolic materials for PTE. The most common embolization techniques were B-RTO with EOI. This procedure involves occlusion of blood flow of PSS by dilation of a balloon catheter, and injection of EOI. We sometimes feared that embolization using too much EOI would induce complications. Therefore, we defined the amount of EOI as below 0.4 mL/kg bodyweight. The required amount of EOI generally varies according to the diameter and length of the shunt. A case of technical failure in the first session had a too long and large shunt. Although we tried to inject EOI under balloon inflation in this case (patient no. 3), the sclerosing agent did not stay in the PSS in spite of also using coils. After the experience of this case, we used NBCA in addition to coils for the complete blockage, to fill the gaps of coils in the longer or larger PSS (patient no. 9).[23,24]

Usually, B-RTO leads to thrombus formation in whole shunts. On the other hand, we embolized short-range PSS when NBCA with coil embolization was adopted in two patients (patients no. 9 and 13). Therefore, we question whether it is necessary that the whole shunt between the in- and outflow vessel should be embolized in PSE with PSS like B-RTO. From our limited experience, whole shunt occlusion may not always be necessary. We think cases with gastric varices

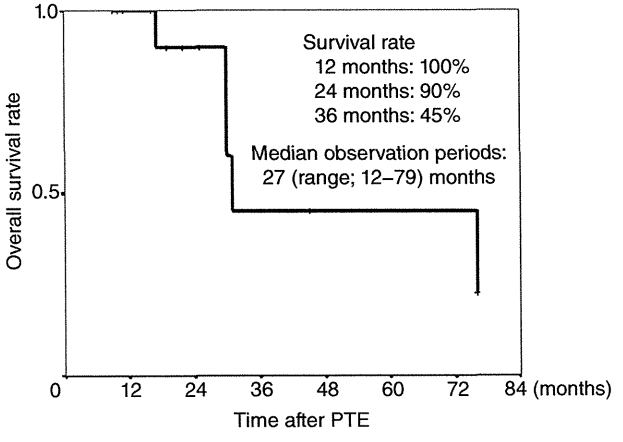

Figure 6 Kaplan–Meier analyses of overall cumulative survival rate. PTE, percutaneous transvenous embolization.

should be embolized up to the inflow vessel beyond varices to prevent variceal rupture in the future.

If embolization of the draining vessel alone would lead to a good outcome, the amount of EOI could be lessened. When coil and NBCA are combined, the amount of EOI could be further lessened. We think that embolization of the draining vessel alone may achieve good outcomes. Recently, embolization with foam has been reported in B-RTO. This may reduce the amount of EOI.[31,32]

When the length of PSS is too short, it is difficult to use EOI because of the possibility of overflow into the portal vein. In such cases, embolization with a combination coils and NBCA may be a safe and useful technique.

The median follow-up period in this study was 27 months, and all patients were observed for more than 1 year. None of the routes of embolized PSS showed re-canalization on CT examinations. We think that once PSS was embolized, the route itself maintained occlusion. If PSE recurred after clinical success, we should consider the development of another PSS route like in patient no. 13. It should be ideal that all major shunts are completely embolized step by step to achieve good outcome in long-term periods.

Major complications were not encountered in any of the patients. PTE may be safe for PSS treatment. However, minor complications occurred frequently. Fever elevation, gross hematuria and jaundice tended to occur when EOI was used (Table 3). We think that complications related to PTE can be mainly attributed to EOI in our experience. The least possible amount of EOI is preferable.

In addition, we have to be careful of elevation of portal venous flowing blood aggravating complications of portal hypertension, including worsening of esophageal varices, ascites and splenomegaly after PTE. PTE may be generally contraindicated for patients with severe liver dysfunctions.[33] Child–Pugh score is evaluated by five clinical measures: (i) serum albumin; (ii) total bilirubin; (iii) prothrombin time activity percentage; (iv) ascites; and (v) encephalopathy. This score was not significantly changed in our patient before (median, 8; range; 6–10) and after PTE (median, 7; range, 5–11). From our experience, patients with a score of less than 10 points may tolerate PTE procedures. We experienced worsening of esophageal varices in 28.6% (4/14 patients), but all of these worsening varices were resolved by endoscopic therapy. For reduction of portal venous pressure after PTE, splenic embolization or splenectomy would be helpful after PTE.[8,34,35] We consider that the analysis of hepatic vein wedge pressure (i.e. portal vein pressure) before and immediately after PTE can be a predictive factor of aggravating complications of portal hypertension in the follow-up period.[36]

Five of fourteen patients died during the follow-up periods (median; 27 months). Although one of five patients died of hepatic failure, we consider that this hepatic failure was not related to PTE because the time of death was 30 months after PTE. The cause of death also was unrelated to PTE in the other four patients.

A limitation of this study was that it included only 14 cases and was retrospective. More cases and longer observation would be necessary for patients with PSE to establish the efficient PTE in the future.

In conclusion, PTE could be one of the useful treatment options for PSE caused by PSS. Patient status after successful PTE can be improved by PSE in the long term, but we should be careful of development of esophageal varices in particular.

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LETTER TO THE EDITOR

Availability of monitoring serum HBV DNA plus RNA during nucleot(s)ide analogue therapy

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We appreciate the comments by Kurosaki et al. on the article entitled "Serum HBV RNA and HBeAg are useful markers for the safe discontinuation of nucleot(s)ide analogue (NUC) treatments in chronic hepatitis B patients" [1]. They raised three important questions: (1) whether HBV DNA levels measured by transcription-mediated amplification and hybridization (TMA-HPA) can be used as an alternative to HBV DNA plus RNA levels measured by RT-PCR; (2) whether post-treatment monitoring of serum HBV DNA plus RNA might serve as a predictor of safe discontinuation after long term NUC; and (3) whether serum HBV DNA plus RNA titer is a predictor of favorable response to sequential interferon therapy.

The presence of HBV RNA in serum is an indicator of ongoing transcription of the HBV pregenome from cccDNA in hepatocytes and may occur even when production of mature HBV particles is effectively suppressed by inhibition of reverse transcription by NUC. As we previously reported, lamivudine resistant strains emerge more easily under such conditions [2], but HBV RNA gradually decreases under continued suppression of reverse transcription and generally becomes undetectable in patients following a year of NUC treatment.

The first question Kurosaki et al. was whether HBV DNA titers measured by TMA-HPA assay, which actually represent HBV DNA plus RNA titers, can be used as an alternative to HBV DNA plus RNA measured by RT-PCR. As we showed in our previous report [2], levels obtained by TMA-PHA assay correlated well with those obtained by RT-PCR during NUC therapy ($r = 0.955$, $P < 0.0001$) [2]. Therefore, measurement of TMA-PHA is a reasonable alternative to RT-PCR. Although the sensitivity of HBV nucleic acids by TMA-HPA assay is lower than RT-PCR, measurement of HBV nucleic acids may provide useful information, especially for those patients who started NUC therapy with high pretreatment HBV DNA levels. RT-PCR is more useful in patients who had relatively lower HBV levels at the beginning of NUC therapy.

The second question was whether monitoring of serum HBV DNA plus RNA at the end of treatment serves as a predictor of safe discontinuation after long term NUC. We found that HBV RNA can be detected in patients who became negative for HBV DNA after long term NUC therapy, and measurement of HBV RNA in patients receiving long term NUC therapy may yield important insight into the risk of reactivation of HBV if NUC therapy is discontinued. However, we have not analyzed enough such patients, and a prospective study is necessary to evaluate the predictive value of HBV RNA plus RNA measurement.

The third question was whether serum HBV DNA plus RNA titer is a predictor of favorable response to sequential NUC and interferon therapy. The mechanisms of these drugs is different, and interferon is not associated with serum HBV RNA because it does not disturb reverse

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transcription but instead suppresses HBV transcription in hepatocytes. In our previous study [3], HBV RNA was negative before administration of NUC and became positive soon after the beginning of NUC therapy, peaking at weeks two to four and then gradually decreasing. We assumed that, after HBV RNA levels have been reduced during long term NUC therapy, HBV RNA should become undetectable during interferon therapy [3]. We tried to assess the predictive effect of HBV RNA titer immediately prior to interferon administration in patients who received sequential therapy, but, incidentally, HBV RNA was undetectable in all patients just before interferon treatment [3]. As we did not show results for sequential therapy in our study in Journal of Gastroenterology [1], results of the 26 patients (20 males, 6 females) who underwent sequential therapy patients in that study are described below. Ten patients were positive for HBeAg at the end of NUC therapy. HBV DNA rebound was observed in 13 patients within 24 weeks after discontinuation of NUC therapy, and ALT rebound occurred in 9 patients. HBV DNA rebound was significantly associated with serum HBV DNA plus RNA titer following 3 months of NUC treatment ($P = 0.029$, Mann–Whitney U test), and ALT rebound was significantly associated with serum HBV DNA titer and DNA plus RNA titer following 3 months of NUC treatment ($P = 0.041$, $P = 0.016$, respectively, Mann–Whitney U test) and the existence of HBeAg at the end of NUC treatment ($P = 0.009$, Fisher's exact test). Although it is necessary to confirm these results in a large, prospective study, we conclude from these results that HBV RNA plus DNA is a predictor for sequential therapy.

Due to the complicated nature of chronic HBV infection and immunological reaction of the host, it is difficult to completely predict the effect of any type of therapy. Further study should be done to identify conditions for safe discontinuation of NUC because otherwise patients must continue lifelong NUC therapy. We thank Kurosaki et al. for their helpful comments and appreciate the opportunity to respond to their questions.

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Original Article

Utility of controlled attenuation parameter measurement for assessing liver steatosis in Japanese patients with chronic liver diseases

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Aim: Steatosis is a common histological feature of chronic liver disease, especially alcoholic and non-alcoholic fatty liver disease, as well as chronic hepatitis C. A recent study showed that evaluating the controlled attenuation parameter (CAP) with transient elastography was an efficient way of non-invasively determining the severity of hepatic steatosis. The objective of this study was to prospectively evaluate the utility of CAP for diagnosing steatosis in patients with chronic liver disease.

Methods: One hundred and fifty-five consecutive patients with suspected chronic liver disease underwent steatosis diagnosis using CAP, blood sample analyses, computed tomography for assessing the liver/spleen ratio and liver biopsy. Steatosis was graded according to the percentage of fat-containing hepatocytes: S0, less than 5%; S1, 5–33%; S2, 34–66%; and S3: more than 66%.

Results: The CAP was significantly correlated with steatosis grade, and there were significant differences between the CAP value of the S0 patients and those of the patients with other grades of steatosis. S0 and S1–3 hepatic steatosis were considered to represent mild and significant steatosis, respectively. The CAP values of the patients with mild and significant steatosis were significantly different ($P < 0.0001$). The area under the receiver–operator curve (AUROC) value of the CAP for diagnosing significant steatosis was 0.878 (95% confidence interval, 0.818–0.939), and the optimal CAP cut-off value for detecting significant steatosis was 232.5 db/m. In multivariate analysis, the CAP ($P = 0.0002$) and the liver to spleen ratio ($P = 0.004$) were found to be significantly associated with significant steatosis.

Conclusion: The CAP is a promising tool for rapidly and non-invasively diagnosing steatosis.

Key words: controlled attenuation parameter, FibroScan, liver steatosis, non-invasively diagnose

INTRODUCTION

THE INCIDENCE OF obesity has markedly increased in developed countries in the past few decades. Due to the Westernization of lifestyles in Japan, the frequency of patients presenting with non-alcoholic fatty liver disease (NAFLD) has gradually increased, and NAFLD/non-alcoholic steatohepatitis (NASH) is estimated to affect 10 million people in the general population.[1,2] NAFLD is one of the clinical consequences of obesity and can progress to NASH, ultimately leading to cirrhosis, hepatocellular carcinoma and end-stage liver failure.[3,4]

Liver steatosis is considered to be a risk factor for treatment failure among patients with chronic viral hepatitis, such as that caused by hepatitis B virus (HBV) or hepatitis C virus (HCV).[5] In addition, previous studies demonstrated that the frequency of liver steatosis was significantly lower in hepatitis C patients who

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achieved a sustained virological response (SVR).[6–11] Although the incidence of liver transplantation for end-stage liver disease is increasing, there is a shortage of organs for living donor liver transplantation.[12,13] Accordingly, it is important to properly estimate the degree of liver steatosis in potential donor livers in order to ensure the success of liver transplantation and donor safety.

Liver biopsy is the current gold standard for evaluating steatosis and other histological lesions;[3,4,14] however, it is invasive, subject to sampling error and is sometimes painful.[15,16] To avoid unnecessary biopsy examinations, various non-invasive methods have been developed for the assessment of hepatic steatosis.[17] As fat affects ultrasound propagation, a novel attenuation parameter has been developed to detect and quantify steatosis. This parameter, which is called the controlled attenuation parameter (CAP) because it specifically targets the liver, is based on the ultrasonic properties of the reflected radio frequency signals acquired by the FibroScan M probe (Echosens, Paris, France). Although many reports have demonstrated the utility of the CAP to determine the extent of a patient's liver steatosis,[18–20] its utility for assessing chronic liver disease in Japanese patients is unknown.

The primary objective of our study was to validate the ability of the CAP to detect and quantify steatosis. The secondary objective was to determine whether steatosis could be assessed simultaneously using the FibroScan M probe in patients with biopsy-proven chronic hepatitis due to any cause.

METHODS

Study population

ONE HUNDRED AND fifty-five consecutive patients with suspected chronic hepatitis due to any etiology who underwent liver biopsy and an ultrasound examination with the FibroScan M probe on the same day to calculate their CAP and liver stiffness measurement (LSM) values were enrolled. The patients were recruited at our institution between April and December 2012.

LSM and CAP measurement

After performing conventional ultrasonography to search for hepatocellular carcinoma, the tip of the transducer probe was placed on the patient's skin between the ribs over the right lobe of the liver with the patient lying in the dorsal decubitus position. All patients had their CAP measured using a standard 3.5-MHz M probe. In a preliminary retrospective study, in which the CAP was assessed in 115 patients with chronic liver disease due to various etiologies, the CAP performed well during the detection and semiquantification of steatosis.[18] The LSM was determined using a FibroScan M probe, a Vibration-Controlled Transient Elastography (VCTE; Echosens) device that is designed to measure liver stiffness. Briefly, the VCTE system generates a 50-Hz shear wave that is longitudinally polarized along the ultrasound axis.[21,22] The median value of 10 measurements performed at depths ranging 25–65 mm was adopted as the final liver stiffness value and was expressed in kPa. Only results derived from five valid shots and displaying an interquartile range (IQR)/median liver stiffness ratio of less than 30% were included. The CAP was designed to measure liver ultrasonic attenuation (along the go and return path) at 3.5 MHz using the signals acquired by the FibroScan M probe.[18] The CAP uses a sophisticated guidance process based on VCTE. In brief, the CAP is based on validated measurements, which are subject to the same criteria as the LSM and are obtained from the same signals. Therefore, the LSM and CAP were obtained simultaneously and in the same volume of liver parenchyma (i.e. at depths of between 25 and 65 mm). The median of the individual CAP values was used as the final CAP value, which was expressed in dB/m. The ratio of the IQR of the CAP values to the median CAP value (IQR/Mcap) was calculated as an indicator of variability.[18–20]

Clinical and laboratory evaluations

Biological and clinical parameters were assessed during liver biopsy. The following data were recorded: age; sex; etiology; height; bodyweight; body mass index (BMI); aspartate aminotransferase, alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), total bilirubin, albumin, triglyceride, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, fasting glucose (FBS) and hemoglobin A1c (HbA1c) levels; prothrombin time; and platelet count. All blood sample analyses were performed in our hospital laboratory. Liver density was assessed using the ratio of the mean computed tomography (CT) attenuation value of the liver (in Hounsfield units; HU) to that of the spleen (L/S ratio), which was evaluated using abdominal CT.

Liver biopsy

Liver biopsy was performed by senior surgeons using a 1.2 mm/1.6 mm diameter Menghini needle (Surecut needle, Create Medic Co. Ltd, Japan). The liver speci-

mens measured more than 20 mm in length and were fixed, embedded in paraffin, and then stained with hematoxylin and Masson-trichrome. One experienced pathologist analyzed all of the biopsies independently without knowledge of the clinical data. Steatosis was graded according to the method of Kleiner *et al.*[23] as: S0, steatosis in less than 5% of hepatocytes; S1, 5–33%; S2, 34–66%; and S3, more than 66%

Statistical analyses

The relationships between the CAP and clinical or morphological parameters were evaluated using Spearman's rank correlation coefficient. Multivariate analysis was performed using multiple linear regression to investigate the effects of fibrosis stage, activity grade and steatosis grade on liver stiffness and the CAP. Box plots were used to assess the utility of the non-invasive methods for differentiating between each grade of steatosis. Area under the receiver–operator curve (AUROC) values were computed as well as their 95% confidence intervals (CI) using the Mann–Whitney *U*-test statistic according to the method proposed by Hanley and McNeil.[24] The cut-off value that maximized the accuracy, sensitivity, and negative and positive predictive values of the CAP for diagnosing significant steatosis was computed. All statistical analyses were performed using the SPSS software ver. 18 (SPSS, Chicago, IL, USA). Statistical results associated with *P*-values of less than 0.05 were considered significant.

RESULTS

Patient characteristics

THE BASELINE CHARACTERISTICS of the 155 patients are shown in Table 1. The median age was 55.0 years (range, 24–91), and 92 patients were male. Etiologies of chronic liver diseases were chronic hepatitis B ($n = 17$), chronic hepatitis C ($n = 58$), NASH ($n = 40$), unknown etiology ($n = 35$) and normal liver ($n = 5$). Their median BMI was 24.4 kg/m^2 (range, 15.4–39.2). The patients' median CAP value was 231.0 dB/m (range, 100–400) and their median LSM was 10.7 kPa (range, 2.60–75.0). CT examinations were available in 97 patients, and the median L/S ratio of these patients was 1.05 (range, −0.144 to 2.03).

CAP values for steatosis assessment

The CAP values of each steatosis grade are shown in Figure 1. The median (25–75% quartiles) CAP values for each steatosis grade were: 202.1 dB/m (range, 100–298) for S0, 279.5 dB/m (range, 179–400) for S1, 297.7 dB/m (range, 162–367) for S2 and 323.0 dB/m (range, 290–345) for S3. There were significant differences between the CAP values for S0 and S1 ($P < 0.0001$), S0 and S2 ($P < 0.0001$), and S0 and S3 ($P < 0.0001$). A box plot of the CAP values of the patients with mild (steatosis affecting <5% of hepatocytes) and significant (steatosis affecting ≥5% of hepatocytes) hepatic steatosis is shown in Figure 2. The median CAP value for mild hepatic steatosis was 202.1 dB/m, and that for significant hepatic steatosis was 285.1 dB/m. There was a significant difference between the CAP values for mild and significant hepatic steatosis ($P < 0.0001$).

The AUROC of the CAP for differentiating between mild and significant steatosis is shown in Figure 3. The

Table 1 Bioclinical and historical characteristics of the patients

Characteristics	Patient data
No. of patients	155
Age (years)	55.0 (24–91)
Sex (male/female)	92/63
Etiology (B/C/NASH/others)	17/58/40/40
Height (m)	1.61 (1.40–1.79)
Bodyweight (kg)	64.0 (39.5–117.2)
Body mass index (kg/m^2)	24.4 (15.4–39.2)
AST (IU/L)	52.0 (14–467)
ALT (IU/L)	64.2 (7–657)
Total bilirubin (mg/dL)	1.0 (0.3–9.3)
Serum albumin (g/dL)	4.2 (2.8–5.4)
Prothrombin (%)	93.7 (43–140)
Platelet count (×104/μL)	19.3 (6.2–54.3)
Triglycerides (mg/dL)	113.5 (23–479)
Total cholesterol (mg/dL)	182.9 (68–336)
High-density lipoprotein cholesterol (mg/dL)	60.5 (12–179)
Low-density lipoprotein cholesterol (mg/dL)	113.6 (26–204)
Fasting blood sugar (mg/dL)	108.9 (21–179)
HbA1c (NGSP, %)	6.0 (4.8–10.1)
Controlled attenuation parameter (CAP, dB/m)	231.0 (100–400)
Liver stiffness measurements (LSM, kPa)	10.7 (2.60–75.0)
L/S ratio	1.05 (−0.144 to 2.03)

All data are median (range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; B, HBs antigen positive; HbA1c, hemoglobin A1c; C, HCV antibody positive; L/S, liver/spleen; NASH, non-alcoholic steatohepatitis; NGSP, National Glycohemoglobin Standardization Program.

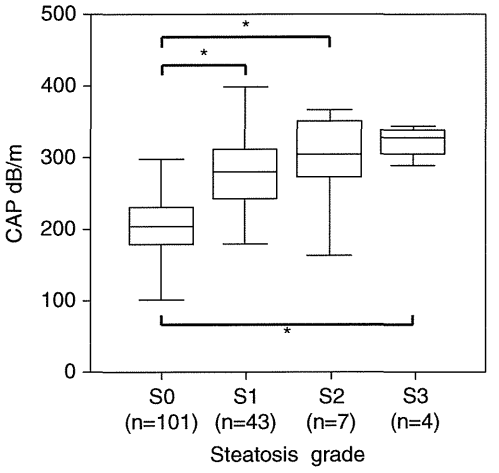

Figure 1 Distribution of controlled attenuation parameter (CAP) for each steatosis grade. The bottom and top of each box represent the 25th and 75th percentiles, giving the interquartile range. The line through the box indicates the median, and the error bars indicate the 10th and 90th percentiles. *$P < 0.0001$.

CAP displayed an AUROC value of 0.878 (95% CI, 0.818–0.939) for diagnosing significant hepatic steatosis. The optimal CAP cut-off value for differentiating between mild and significant hepatic steatosis was 232.5 dB/m, which produced sensitivity and specificity values of 87.0% and 77.2%, respectively, as well as a positive predictive value (PPV) of 75.2% and a negative predictive value (NPV) of 87.0%. The AUROC based on the individual etiologies were shown in Supporting Information Figure S1.

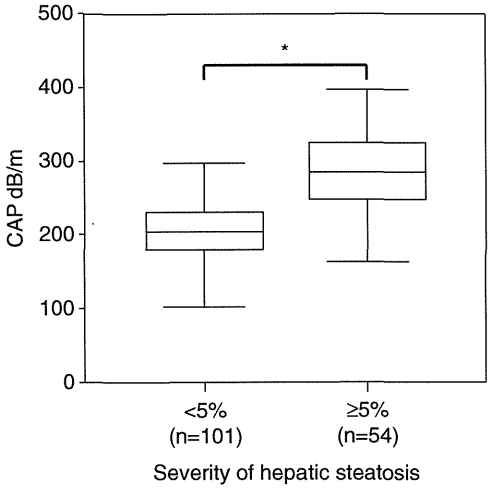

Figure 2 Box plot of controlled attenuation parameter (CAP) in hepatic steatosis according to severity <5% and ≥5%. There is significant correlation between CAP and frequency of steatosis. *$P < 0.0001$.

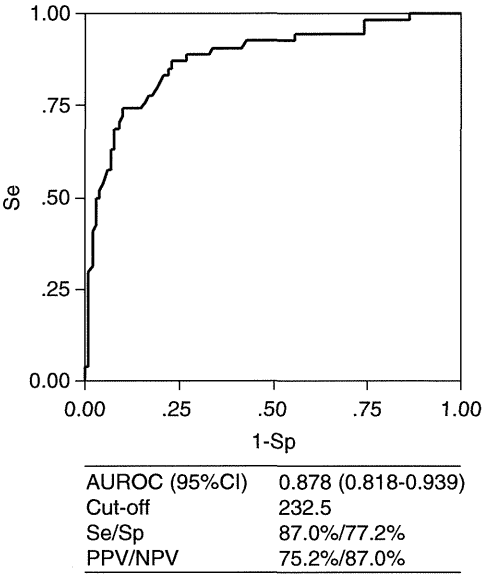

Figure 3 AUROC to compare the diagnostic accuracy of liver steatosis (<5% and ≥5%) assessed by controlled attenuation parameter. AUROC, area under the receiver-operator curve; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity.

The results of our univariate analysis of the factors associated with significant steatosis are shown in Table 2. Among the analyzed factors, BMI, cholinesterase, the CAP value and L/S ratio displayed the most significant associations with significant steatosis ($P < 0.0001$). ALT ($P = 0.0001$), triglyceride ($P = 0.002$), HbA1c ($P = 0.002$), alkaline phosphatase ($P = 0.007$), white blood cell ($P = 0.020$), platelet count ($P = 0.020$), GGT ($P = 0.028$), FBS ($P = 0.036$) and total cholesterol ($P = 0.043$) also displayed significant associations with significant hepatic steatosis. In the multivariate analysis, only the CAP value (odds ratio, 27.656; 95% CI, 4.762–160.622; $P = 0.0002$) and L/S ratio (odds ratio, 10.881; 95% CI, 2.101–56.361; $P = 0.004$) were significantly associated with significant steatosis (Table 3).

DISCUSSION

IN JAPAN, MUCH attention has been paid to HBV/HCV-infected patients over the past few decades because there are high numbers of carriers of these viruses in Japan, and most cases of cirrhosis and hepa-

Table 2 Factors associated with steatosis ≥5% on liver biopsy (univariate analysis)

Variable		Severity of hepatic steatosis				*P*-value
		<5%		≥5%		
		n	Mean ± SD	*n*	Mean ± SD	
Age	<60/≥60	50/51	45.8 ± 9.8/66.6 ± 6.1	34/20	44.7 ± 10.3/65.6 ± 4.8	0.129
Sex	Female/male	44/57		19/35		0.391
BMI (kg/m^2)	<25/≥25	74/27	21.6 ± 2.0/26.8 ± 1.9	18/36	23.1 ± 2.0/29.3 ± 4.0	<0.0001
AST (IU/L)	<33/≥33	50/51	25.1 ± 5.0/90.9 ± 99.5	19/35	24.1 ± 5.6/49.2 ± 17.7	1.000
ALT (IU/L)	<35/≥35	51/50	24.0 ± 7.0/113.8 ± 114.0	12/42	24.9 ± 8.0/65.0 ± 22.7	0.0001
ALP (IU/L)	<359/≥359	68/33	223 ± 60/587 ± 243	47/7	222 ± 55/525 ± 189	0.007
GGT (IU/L)	<41/≥41	53/48	23.4 ± 8.6/281 ± 522	18/36	26.0 ± 9.6/103 ± 73	0.028
Cholinesterase (IU/L)	<300/≥300	69/32	224 ± 58/339 ± 35	16/38	228 ± 56/381 ± 50	<0.0001
Total bilirubin (mg/dL)	<1.2/≥1.2	83/18	0.74 ± 0.19/2.48 ± 1.9	47/7	0.73 ± 0.19/1.51 ± 0.38	0.498
Serum albumin (mg/dL)	<4.3/≥4.3	51/50	3.73 ± 0.39/4.50 ± 0.26	24/30	3.97 ± 0.26/4.64 ± 0.31	0.503
Prothrombin (%)	<70/≥70	5/96	59.8 ± 10.5/94.7 ± 12.8	2/52	57.5 ± 5.0/96.4 ± 10.9	1.000
White blood cell (/μL)	<4000/≥4000	25/76	3318 ± 578/6143 ± 1806	5/49	3522 ± 485/6929 ± 2836	0.020
Platelet count (/μL)	<22×10^4/≥22×10^4	75/26	15.4 ± 4.1/27.4 ± 5.2	30/24	15.4 ± 4.1/27.4 ± 6.6	0.020
Triglyceride (mg/dL)	<149/≥149	81/13	85.9 ± 28.7/182 ± 46.5	32/19	82.5 ± 29.7/236 ± 83.8	0.002
Total cholesterol (mg/dL)	<179/≥179	56/45	149 ± 25/213 ± 33	20/34	162 ± 15/212 ± 22	0.043
FBS (mg/dL)	<109/≥109	65/33	97.7 ± 6.6/141.8 ± 37.0	25/27	99.3 ± 6.7/133.9 ± 32.0	0.036
HbA1c (NGSP, %)	<6.2/≥6.2	63/14	5.6 ± 3.7/6.5 ± 0.6	26/21	5.2 ± 0.3/6.9 ± 1.3	0.002
CAP (dB/m)	<232.5/≥232.5	76/25	182 ± 34/263 ± 31	7/47	196 ± 24/298 ± 41	<0.0001
LSM (kPa)	<10.7/≥10.7	77/24	6.0 ± 1.8/29.0 ± 22.0	36/18	6.1 ± 2.1/15.5 ± 4.6	0.255
L/S ratio	≥1.1/ < 1.1	40/11	1.27 ± 0.16/1.01 ± 0.07	13/33	1.18 ± 0.08/0.74 ± 0.29	<0.0001

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FBS, fasting blood sugar; CAP, controlled attenuation parameter; GGT, γ-glutamyltransferase; HbA1c, hemoglobin A1c; L/S, liver/spleen; LSM, liver stiffness measurement; NGSP, National Glycohemoglobin Standardization Program; SD, standard deviation.

tocellular carcinoma in Japan are associated with persistent HBV or HCV infection.[25] In recent years, NAFLD has become a major social problem in Japan due to the Westernization of lifestyles and the increasing rates of obesity and diabetes.[26] Approximately 30% of NAFLD patients are considered to progress to NASH, a more severe form of NAFLD, which leads to more advanced fibrosis and ultimately cirrhosis.[27] Among chronic viral hepatitis patients, liver steatosis is a risk factor for infection and treatment failure.[5] Chronic HCV infection is associated with fatty liver changes, and HCV patients display a higher incidence of fatty changes than patients with other chronic liver dysfunctions.[28,29] Furthermore, Okanoue *et al.* demonstrated that the frequency of liver steatosis was significantly lower in hepatitis C patients who achieved an SVR.[11] Therefore, it is important to diagnose and evaluate the severity of steatosis to improve its treatment and prognosis. Liver biopsy is the current gold standard for evaluating steatosis and other histological lesions;[3,4,14] however, liver biopsy can be affected by sampling error,[15,16] is an invasive and often painful procedure, and can result in severe complications.[30,31] Moreover, the repetition of liver biopsy to monitor changes in steatosis is difficult. In light of these obstacles, various non-invasive methods have been developed for the assessment of hepatic histology, particularly fibrosis.[17,32] Steatosis can also be diagnosed by non-invasive means and is mainly diagnosed using conventional imaging techniques, for example, CT, multiple resonance imaging (MRI), magnetic resonance

Table 3 Factors associated with steatosis ≥5% on liver biopsy (multivariate analysis)

Variable	Odds ratio	95% confidence interval	*P*-value
CAP ≥232.5 (dB/m)	27.656	4.762–160.622	0.0002
L/S ratio <1.1	10.881	2.101–56.361	0.004

Factors: body mass index, ≥25; alanine aminotransferase, ≥35; alkaline phosphatase, ≥359; γ-glutamyltransferase, ≥41; cholinesterase, ≥300; white blood cell, ≥4000; platelet count, ≥20 × 10^4; triglyceride, ≥149; total cholesterol, ≥179; fasting blood sugar, ≥109; hemoglobin A1c, ≥5.7; controlled attenuation parameter (CAP), ≥243.5; liver/spleen (L/S) ratio <1.1.