References

[1] W.C. Maddrey, Hepatitis B: an important public health issue, J. Med. Virol. 61 (2000) 362–366.

[2] Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium, J. Viral Hepat. 6 (1999) 35–47.

[3] R.P. Beasley, Hepatitis B virus. The major etiology of hepatocellular carcinoma, Cancer 61 (1988) 1942–1956.

[4] D.F. Mercer, D.E. Schiller, J.F. Elliott, D.N. Douglas, C. Hao, A. Rinfret, W.R. Addison, K.P. Fischer, T.A. Churchill, J.R. Lakey, D.L. Tyrrell, N.M. Kneteman, Hepatitis C virus replication in mice with chimeric human livers, Nat. Med. 7 (2001) 927–933.

[5] C. Tateno, Y. Yoshizane, N. Saito, M. Kataoka, R. Utoh, C. Yamasaki, A. Tachibana, Y. Soeno, K. Asahina, H. Hino, T. Asahara, T. Yokoi, T. Furukawa, K. Yoshizato, Near completely humanized liver in mice shows human-type metabolic responses to drugs, Am. J. Pathol. 165 (2004) 901–912.

[6] H. Azuma, N. Paulk, A. Ranade, C. Dorrell, M. Al-Dhalimy, E. Ellis, S. Strom, M.A. Kay, M. Finegold, M. Grompe, Robust expansion of human hepatocytes in Fah-/-/Rag2-/-/Il2rg-/-mice, Nat. Biotechnol. 25 (2007) 903–910.

[7] K.D. Bissig, T.T. Le, N.B. Woods, I.M. Verma, Repopulation of adult and neonatal mice with human hepatocytes: a chimeric animal model, Proc. Natl. Acad. Sci. USA 104 (2007) 20507–20511.

[8] H. Suemizu, M. Hasegawa, K. Kawai, K. Taniguchi, M. Monnai, M. Wakui, M. Suematsu, M. Ito, G. Peltz, M. Nakamura, Establishment of a humanized model of liver using NOD/Shi-scid IL2Rgnull mice, Biochem. Biophys. Res. Commun. 377 (2008) 248–252.

[9] Y.P. de Jong, C.M. Rice, A. Ploss, New horizons for studying human hepatotropic infections, J. Clin. Invest. 120 (2010) 650–653.

[10] Z. He, H. Zhang, X. Zhang, D. Xie, Y. Chen, K.J. Wangensteen, S.C. Ekker, M. Firpo, C. Liu, D. Xiang, X. Zi, L. Hui, G. Yang, X. Ding, Y. Hu, X. Wang, Liver xenorepopulation with human hepatocytes in Fah-/-Rag2-/-mice after pharmacological immunosuppression, Am. J. Pathol. 177 (2010) 1311–1319.

[11] K.D. Bissig, S.F. Wieland, P. Tran, M. Isogawa, T.T. Le, F.V. Chisari, I.M. Verma, Human liver chimeric mice provide a model for hepatitis B and C virus infection and treatment, J. Clin. Invest. 120 (2010) 924–930.

[12] M. Tsuge, N. Hiraga, H. Takaishi, C. Noguchi, H. Oga, M. Imamura, S. Takahashi, E. Iwao, Y. Fujimoto, H. Ochi, K. Chayama, C. Tateno, K. Yoshizato, Infection of human hepatocyte chimeric mouse with genetically engineered hepatitis B virus, Hepatology 42 (2005) 1046–1054.

[13] E. Ohara, N. Hiraga, M. Imamura, E. Iwao, N. Kamiya, I. Yamada, T. Kono, M. Onishi, D. Hirata, F. Mitsui, T. Kawaoka, M. Tsuge, S. Takahashi, H. Abe, C.N. Hayes, H. Ochi, C. Tateno, K. Yoshizato, S. Tanaka, K. Chayama, Elimination of hepatitis C virus by short term NS3–4A and NS5B inhibitor combination therapy in human hepatocyte chimeric mice, J. Hepatol. 54 (2011) 872–878.

[14] N. Hiraga, H. Abe, M. Imamura, M. Tsuge, S. Takahashi, C.N. Hayes, H. Ochi, C. Tateno, K. Yoshizato, Y. Nakamura, N. Kamatani, K. Chayama, Impact of viral amino acid substitutions and host interleukin-28b polymorphism on replication and susceptibility to interferon of hepatitis C virus, Hepatology 54 (2011) 764–771.

[15] M. Hasegawa, K. Kawai, T. Mitsui, K. Taniguchi, M. Monnai, M. Wakui, M. Ito, M. Suematsu, G. Peltz, M. Nakamura, H. Suemizu, The reconstituted 'humanized liver' in TK-NOG mice is mature and functional, Biochem. Biophys. Res. Commun. 405 (2011) 405–410.

[16] H. Yamazaki, H. Suemizu, N. Murayama, M. Utoh, N. Shibata, M. Nakamura, F.P. Guengerich, In vivo drug interactions of the teratogen thalidomide with midazolam: heterotropic cooperativity of human cytochrome P450 in humanized TK-NOG mice, Chem. Res. Toxicol. 26 (2013) 486–489.

[17] H. Yamazaki, H. Suemizu, M. Shimizu, S. Igaya, N. Shibata, M. Nakamura, G. Chowdhury, F.P. Guengerich, In vivo formation of dihydroxylated and glutathione conjugate metabolites derived from thalidomide and 5-Hydroxythalidomide in humanized TK-NOG mice, Chem. Res. Toxicol. 25 (2012) 274–276.

[18] Y. Hu, M. Wu, T. Nishimura, M. Zheng, G. Peltz, Human pharmacogenetic analysis in chimeric mice with 'humanized livers', Pharmacogenet. Genomics 23 (2013) 78–83.

[19] N. Hiraga, M. Imamura, M. Tsuge, C. Noguchi, S. Takahashi, E. Iwao, Y. Fujimoto, H. Abe, T. Maekawa, H. Ochi, C. Tateno, K. Yoshizato, A. Sakai, Y. Sakai, M. Honda, S. Kaneko, T. Wakita, K. Chayama, Infection of human hepatocyte chimeric mouse with genetically engineered hepatitis C virus and its susceptibility to interferon, FEBS Lett. 581 (2007) 1983–1987.

[20] T. Vanwolleghem, L. Libbrecht, B.E. Hansen, I. Desombere, T. Roskams, P. Meuleman, G. Leroux-Roels, Factors determining successful engraftment of hepatocytes and susceptibility to hepatitis B and C virus infection in uPA-SCID mice, J. Hepatol. 53 (2010) 468–476.

[21] M. Ito, H. Hiramatsu, K. Kobayashi, K. Suzue, M. Kawahata, K. Hioki, Y. Ueyama, Y. Koyanagi, K. Sugamura, K. Tsuji, T. Heike, T. Nakahata, NOD/SCID/gamma(c) (null) mouse: an excellent recipient mouse model for engraftment of human cells, Blood 100 (2002) 3175–3182.

[22] P. Meuleman, L. Libbrecht, R. De Vos, B. de Hemptinne, K. Gevaert, J. Vandekerckhove, T. Roskams, G. Leroux-Roels, Morphological and biochemical characterization of a human liver in a uPA-SCID mouse chimera, Hepatology 41 (2005) 847–856.

[23] X. Wang, H. Willenbring, Y. Akkari, Y. Torimaru, M. Foster, M. Al-Dhalimy, E. Lagasse, M. Finegold, S. Olson, M. Grompe, Cell fusion is the principal source of bone-marrow-derived hepatocytes, Nature 422 (2003) 897–901.

IJC
International Journal of Cancer

Serum interleukin-6 associated with hepatocellular carcinoma risk: A nested case–control study

Waka Ohishi[1], John B. Cologne[2], Saeko Fujiwara[1,3], Gen Suzuki[4], Tomonori Hayashi[5], Yasuharu Niwa[5], Masazumi Akahoshi[6], Keiko Ueda[1], Masataka Tsuge[7] and Kazuaki Chayama[7]

[1] Department of Clinical Studies, Radiation Effects Research Foundation, Hiroshima, Japan
[2] Department of Statistics, Radiation Effects Research Foundation, Hiroshima, Japan
[3] Health Management and Promotion Center, Hiroshima Atomic Bomb Casualty Council, Hiroshima, Japan
[4] International University of Health and Welfare Clinic, Ohtawara, Japan
[5] Department of Radiation Biology and Molecular Epidemiology, Radiation Effects Research Foundation, Hiroshima, Japan
[6] Department of Clinical Studies, Radiation Effects Research Foundation, Nagasaki, Japan
[7] Department of Gastroenterology and Metabolism, Applied Life Sciences, Institute of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

Inflammatory markers have been associated with increased risk of several cancers, including colon, lung, breast and liver, but the evidence is inconsistent. We conducted a nested case–control study in the longitudinal cohort of atomic-bomb survivors. The study included 224 hepatocellular carcinoma (HCC) cases and 644 controls individually matched to cases on gender, age, city and time and method of serum storage, and countermatched on radiation dose. We measured C-reactive protein (CRP) and interleukin (IL)-6 using stored sera obtained within 6 years before HCC diagnosis from 188 HCC cases and 605 controls with adequate volumes of donated blood. Analyses with adjustment for hepatitis virus infection, alcohol consumption, smoking habit, body mass index (BMI) and radiation dose showed that relative risk (RR) of HCC [95% confidence interval (CI)] in the highest tertile of CRP levels was 1.94 (0.72–5.51) compared to the lowest tertile (p = 0.20). RR of HCC (95% CI) in the highest tertile of IL-6 levels was 5.12 (1.54–20.1) compared to the lowest tertile (p = 0.007). Among subjects with BMI > 25.0 kg/m^2, a stronger association was found between a 1-standard deviation (SD) increase in log IL-6 and HCC risk compared to subjects in the middle quintile of BMI (21.3–22.9 kg/m^2), resulting in adjusted RR (95% CI) of 3.09 (1.78–5.81; p = 0.015). The results indicate that higher serum levels of IL-6 are associated with increased HCC risk, independently of hepatitis virus infection, lifestyle-related factors and radiation exposure. The association is especially pronounced among subjects with obesity.

Key words: C-reactive protein, interleukin-6, obesity, hepatocellular carcinoma, nested case–control study

Abbreviations: BMI: body mass index; CI: confidence interval; CRP: C-reactive protein; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; IL-6: interleukin-6; RERF: Radiation Effects Research Foundation; RR: relative risk; SD: standard deviation

Conflict of interest: Nothing to report

Grant sponsor: Japanese Ministry of Education, Culture, Sports, Science and Technology; **Grant number:** 20590672; **Grant sponsor:** U.S. Department of Energy (DOE); **Grant number:** DE-HS0000031; **Grant sponsors:** RERF Research Protocols #2-75 and #1-09, Japanese Ministry of Health, Labour and Welfare (MHLW)
DOI: 10.1002/ijc.28337
History: Received 30 Mar 2013; Accepted 31 May 2013; Online 20 Jun 2013

Correspondence to: Waka Ohishi, MD, PhD, Department of Clinical Studies, Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minami-ku, Hiroshima 732-0815, Japan, Tel.: +81-82-261-9122, Fax: +81-82-261-3259, E-mail: nwaka@rerf.or.jp

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. Chronic infections with hepatitis B virus (HBV) or hepatitis C virus (HCV) are recognized as crucially important risk factors for HCC, whereas an increase of HCC without HBV and HCV infection (non-B, non-C HCC) has been noted recently in Japan.[1,2] Although periodic follow-up with imaging, tumor markers such as alpha-fetoprotein (AFP) and fibrosis markers are recommended, these strategies have not been sufficient for early detection of HCC in chronic liver disease, especially in non-B, non-C liver disease. Therefore, it is necessary to identify biomarkers that may be useful to narrow down a high-risk subgroup for HCC.

A large number of epidemiologic studies have shown that obesity and diabetes mellitus are associated with increased risks of such malignant tumors as colon, prostate and breast, as well as HCC.[3–11] Our earlier study also demonstrated that obesity [body mass index (BMI) > 25.0 kg/m^2] 10 years before HCC diagnosis was significantly associated with increased risk of HCC, independently of HBV and HCV infection, alcohol consumption, smoking habit and radiation exposure.[12] It has been suggested that cell proliferation activity of insulin due to hyperinsulinemia or chronic inflammation may promote

Epidemiology

What's new?

According to previous research, alcohol consumption, obesity, and radiation exposure as well as hepatitis virus infection are all independent risk factors for hepatocellular carcinoma (HCC). Inflammatory markers have also been associated with increased risk of liver cancer, but the evidence is inconsistent. In this nested case-control study in the longitudinal cohort of atomic-bomb survivors, which took into account hepatitis virus infection, lifestyle-related factors, and radiation exposure, elevated IL-6 levels were found to be associated with increased risk of HCC. The findings also indicated that association of IL-6 levels with increased risk of HCC is especially pronounced among subjects with obesity.

carcinogenesis by DNA damage, enhancement of cellular proliferation and inhibition of apoptosis.[4,13] In recent years, some studies have suggested that blood levels of inflammatory markers or cytokines also related to insulin resistance—such as C-reactive protein (CRP), interleukin (IL)-6 and tumor necrosis factor (TNF)—may reveal a biological mechanism by which risks of colon, lung and breast cancers increase,[14–17] but other studies have not supported such associations.[18,19]

Several studies have demonstrated that elevated serum levels of IL-6 are associated with increased risk of HCC in female chronic hepatitis C patients,[20] and that the combination of serum levels of IL-6 and alpha-fetoprotein improves sensitivity in diagnosing HCC or predicting future HCC development in chronic hepatitis B patients.[21] A few experimental studies using a mouse model have demonstrated that estrogen-mediated inhibition of IL-6 production by Kupffer cells reduces liver cancer risk in females,[22] and that obesity-promoted HCC development was bound up with elevated 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 several other cancers,[24,25] it has been suggested that IL-6 and STAT3 may also contribute toward a general enhancement of cancer risk by high BMI.

With the aim of investigating whether serum levels of CRP and IL-6 are associated with risk of HCC and, if so, whether that risk is independent of HBV and HCV infection, alcohol consumption, smoking habit, BMI and radiation exposure, we conducted a nested case–control study using sera collected from a prospective cohort study of atomic-bomb survivors. We subsequently evaluated whether the association between serum IL-6 levels and HCC risk is modified by alcohol consumption, smoking habit, BMI or radiation dose to the liver using analyses based on subgroups of those factors.

Material and Methods

Cohorts

The Atomic Bomb Casualty Commission (ABCC) and its successor, the Radiation Effects Research Foundation (RERF), established the prospective Adult Health Study cohort in 1958, in which more than 20,000 gender-, age- and city-matched proximal and distal atomic-bomb survivors and persons not present in the cities at the time of bombings have been examined biennially in outpatient clinics in Hiroshima and Nagasaki.

Cases and controls

Incident cancer cases were identified through the Hiroshima Tumor and Tissue Registry and Nagasaki Cancer Registry, confirmed and supplemented by additional cases detected *via* pathological review of related diseases.[26] As described in our previous studies,[3,27] 359 primary HCC cases were diagnosed among 18,660 Adult Health Study participants between 1970 and 2002, who visited our outpatient clinics before their diagnosis. Of these, 229 cases had serum samples obtained within 6 years before HCC diagnosis (average: stored sera obtained 1.2 years before diagnosis). After excluding five cases with inadequate stored serum, 224 cases remained for our previous studies. There were no important differences in characteristics such as alcohol consumption, smoking habit, BMI or radiation dose to the liver (among exposed persons) between HCC cases excluded because of nonavailability of stored serum and those included in our study.

As described in our previous studies,[3,27] 644 controls were selected from the at-risk cohort members matched to the case on gender, age, city and time and method of serum storage, and countermatched on radiation dose in nested case–control fashion.[28] Counter matching (to increase statistical efficiency for studying joint effects of radiation and other factors) was performed using four strata based on whole-body (skin) dose: zero dose (<0.0005 Gy), <0.05 Gy, <0.75 Gy and ≥ 0.75 Gy (nonzero categories correspond roughly to tertiles of skin dose among all eligible exposed cases). At the time of each case diagnosis, one control serum was selected at random from each of the three dose strata not occupied by the case in the cohort risk set.

Laboratory tests

Virological assays of HBV and HCV were performed on 211 cases and 640 controls with sufficient stored sera for these assays as previously described.[29,30] HBV infection (HBV+) status was defined as positive for HBsAg or having a high titer of anti-HBc Ab (positive for anti-HBc Ab of samples diluted 200-fold). HCV infection (HCV+) status was defined as positive for HCV RNA. Non-B, non-C status was defined as negative for HBsAg and not having a high titer of anti-HBc Ab (HBV-) as well as negative for HCV RNA (HCV-).

Serum levels of CRP were measured using an autoanalyzer (Hitachi 7180, Hitachi, Tokyo, Japan) and a high-sensitivity assay kit (Nissui Pharmaceutical, Tokyo, Japan) containing anti-CRP monoclonal antibodies. The detection limit of CRP was 0.08 mg/L. The intra-assay variability was determined by assaying two pooled serum samples (mean CRP level: 0.62 and 1.68 mg/L, respectively) 20 times in a single day, and the respective coefficients of variation (CVs) were 1.12 and 0.95%. The interassay variability was determined by assaying two quality control samples (mean CRP level: 2.14 and 4.71 mg/L, respectively) once a day 12 for days; the respective CVs were 4.1 and 1.2%. Serum levels of IL-6 were measured using the multiplex bead array assay on the Luminex Complete System 200 (Luminex Corp., Austin, TX),[31] with MILLIPLEX™ MAP kits (Millipore, Billerica, MA) according to the manufacturer's instructions. Human serum adipokine panel B (HADK2-61K-B) was used for IL-6. The intra-assay variability was determined by assaying two pooled serum samples with and without including a quality control sample (mean IL-6 level: 4.29 and 144.82 pg/mL, respectively) 15 times in a single day, and the respective CVs were 8.6 and 7.5%. The interassay variability was determined by assaying two quality control samples (mean IL-6 level: 31.02 and 171.62 pg/mL, respectively) once a day for 7 days; the respective CVs were 7.9 and 13.7%.

Radiation dose

Radiation dose to the liver was estimated for each subject according to Dosimetry System DS02.[32] A weighted sum of the gamma dose in gray plus ten times the neutron dose in gray was used.

Alcohol consumption, smoking habit and BMI

Self-administrated questionnaires on lifestyle-related factors were given to Adult Health Study participants in 1965 during attendance at the outpatient clinic and in 1978 by mail survey. Information on alcohol consumption was obtained from the 1965 questionnaire when available, with missing data complemented using the 1978 mail survey. Mean ethanol amounts were calculated as grams per day, as previously described.[33] Information on smoking habit was obtained from the 1965 questionnaire. Subjects were categorized as never, current or former smoker. BMI (kg/m^2) was calculated from height and weight measured in the outpatient clinic of the Adult Health Study. Subjects were classified based on BMI quintiles with cut points of 19.5, 21.2, 22.9 and 25.0. Following the recommendations for Asian people by the WHO, the International Association for the Study of Obesity and the International Obesity Task Force,[34] 21.3–22.9 kg/m^2 was considered as normal, 23.0–25.0 kg/m^2 as overweight and >25.0 kg/m^2 as obese.

Ethical consideration

This study (RERF Research Protocol 1-09) was reviewed and approved by the Research Protocol Review Committee and the Human Investigation Committee of RERF.

Statistical analyses

The nested case–control design is analyzed using a partial likelihood method analogous to that used for cohort follow-up studies,[35] which is in practice the same as the conditional binary data likelihood for matched case–control studies[36] except that the subjects (cases and controls) in the study are not completely independent owing to the possibility of repeated selection. Radiation risk was estimated using an excess relative risk (ERR) model (ERR = RR − 1) to conform to other analyses of the atomic-bomb survivor cohort.[3,27,37] Bias in control doses due to selecting controls using countermatching was corrected using weights as described elsewhere.[28] Risks for all other factors were assessed using a log-linear model. In analyses based on continuous values, CRP and IL-6 were transformed using the natural logarithm. Analyses using CRP or IL-6 groups used tertiles computed among controls. A two-degree-of-freedom heterogeneity test was performed by comparing the deviance of the model with tertiles to that without, using the lowest tertile as the comparison group. We fit log-linear regression models for the effect of a 1-standard deviation (SD) increase in IL-6 and tested for interaction with each of the other risk factors individually using the same heterogeneity test, with degrees of freedom depending on the number of categories of the other risk factor; we report the p value for the pairwise test comparing the interaction parameter in the highest to lowest level of each other risk factor. We also assessed various models for log relative risk of HCC with continuous level of IL-6—linear, linear-quadratic and linear spline—using the Akaike information criterion (AIC).[38] Analyses were conducted using Epicure (HIROSOFT International Corp., Seattle, WA).

Results

Characteristics of cases and controls

Table 1 shows characteristics of HCC cases and matched controls. Because of matching, cases and controls were comparable with respect to gender, age, city and time and method of serum storage. Prevalence of HBV and/or HCV infection status in HCC cases is higher than in controls. Compared to the controls, higher proportions of HCC cases had a history of alcohol consumption exceeding 40 g of ethanol per day, were obese (BMI > 25.0 kg/m^2) and were current smokers. Median serum levels of CRP were 0.72 mg/L among HCC cases and 0.59 mg/L among controls. Median serum levels of IL-6 were 4.88 pg/mL among HCC cases and 2.90 pg/mL among controls. HCC cases also received on average higher radiation doses to the liver compared to controls.

Correlations among CRP, IL-6, alcohol, BMI and radiation dose

Table 2 shows Spearman rank-correlation coefficients (r) between serum levels of CRP and IL-6, alcohol consumption,

Table 1. Characteristics of HCC cases and controls

Study variables	HCC cases		Controls	
	Number with complete data	*n* (%)	Number with complete data	*n* (%)
Matched variables				
Age at HCC diagnosis (years)[1]	224	67.6 (10.1)	–	–
Age at serum storage (years)[1]	224	66.4 (10.2)	644	63.7 (9.8)
Gender	224		644	
Male		136 (60.7)		387 (60.1)
Female		88 (39.3)		257 (39.9)
City	224		644	
Hiroshima		155 (69.2)		444 (68.9)
Nagasaki		69 (30.8)		200 (31.1)
Unmatched variables				
Viral etiology	211		640	
HBV−/HCV−		45 (21.3)		579 (90.5)
HBV+ and/or HCV+		166 (78.7)		61 (9.5)
Alcohol consumption (g ethanol/day)	199		577	
None		97 (48.7)		315 (54.6)
0< <40		57 (28.6)		194 (33.6)
≥40		45 (22.6)		68 (11.8)
Smoking habit	199		578	
Never		80 (40.2)		283 (49.0)
Current smoker		107 (53.8)		262 (45.3)
Former smoker		12 (6.0)		33 (5.7)
BMI (kg/m^2) 10 years before diagnosis	210		633	
≤19.5		38 (18.1)		122 (19.3)
19.6–21.2		33 (15.7)		136 (21.5)
21.3–22.9		36 (17.2)		142 (22.4)
23.0–25.0		49 (23.3)		124 (19.6)
>25.0		54 (25.7)		109 (17.2)
Inflammatory markers				
CRP (mg/L), median (IQR)	188	0.72 (0.18, 1.89)	605	0.59 (0.25, 1.52)
IL-6 (pg/mL), median (IQR)	182	4.88 (2.88, 8.77)	589	2.90 (1.53, 5.42)
Radiation dose to the liver (Gy)[1,2]	204	0.46 (0.69)	606	0.34 (0.56)

[1]Mean (SD).
[2]Control values were adjusted for countermatched selection.

BMI 10 years before HCC diagnosis and radiation dose to the liver. Serum levels of CRP were positively correlated with serum levels of IL-6 among both cases (r = 0.46) and controls (r = 0.29). Serum levels of CRP were modestly correlated with BMI among both cases (r = 0.15) and controls (r = 0.28), whereas correlations between serum levels of IL-6 and BMI were not significant among either cases (r = 0.11) or controls (r = 0.06). Neither alcohol consumption nor radiation dose showed any evidence of correlation with either marker.

Risk of HCC according to serum levels of CRP and IL-6

Table 3 shows the association between CRP and HCC risk based on tertiles of serum CRP levels. Analyses with adjustment for HBV and HCV infection, alcohol consumption, smoking habit, BMI 10 years before HCC diagnosis and radiation dose showed that relative risks (RRs) of HCC [95% confidence interval (CI)] in the middle tertile (0.37–0.96 mg/L) and highest tertile (>0.96 mg/L) of CRP levels were 2.11 (0.73–6.54; p = 0.17) and 1.94 (0.72–5.51; p = 0.20), respectively, compared to

Table 2. Spearman rank-correlation coefficients between CRP, IL-6, alcohol, BMI and radiation dose among HCC cases and controls

	CRP		IL-6	
Variables	Correlation	*p* Value	Correlation	*p* Value
HCC cases				
CRP	–	–	–	–
IL-6	0.46	<0.001	–	–
Alcohol consumption (g ethanol/day)	0.01	0.9	−0.02	0.83
BMI 10 years before diagnosis	0.15	0.049	0.11	0.14
Radiation dose to the liver	−0.09	0.26	−0.08	0.30
Controls				
CRP	–	–	–	–
IL-6	0.29	<0.001	–	–
Alcohol consumption (g ethanol/day)	−0.003	0.94	0.05	0.28
BMI 10 years before diagnosis	0.28	<0.001	0.06	0.13
Radiation dose to the liver	−0.02	0.64	−0.06	0.13

Table 3. Relative risks of HCC by tertile of serum levels of CRP

	Tertile of CRP			*p* Value for heterogeneity
	Low < 0.37 mg/L	Middle 0.37–0.96 mg/L	High > 0.96 mg/L	
No. of cases/controls[1]	**49/120**	**29/98**	**59/109**	
Crude RR (95% CI)	1.00	0.64 (0.36–1.15)	1.16 (0.71–1.88)	0.10
p Value	–	0.14	>0.50	
Adjusted RR (95% CI)[2]	1.00	1.54 (0.62–3.92)	1.90 (0.87–4.36)	0.28
p Value	–	0.36	0.11	
Adjusted RR (95% CI)[3]	1.00	2.11 (0.73–6.54)	1.94 (0.72–5.51)	0.32
p Value	–	0.17	0.20	

[1]Number of subjects for whom information available for all factors included in a log-linear model: 137 HCC cases and 327 controls.
[2]Adjusted for HBV/HCV infection, excluding three HBV+/HCV+ individuals.
[3]Adjusted for HBV/HCV infection, alcohol consumption, smoking habit, BMI 10 years before diagnosis and radiation dose to the liver.

Table 4. Relative risks of HCC by tertile of serum levels of IL-6

	Tertile of IL-6			*p* Value for heterogeneity
	Low < 2.01 pg/mL	Middle 2.01–4.46 pg/mL	High > 4.46 pg/mL	
No. of cases/controls[1]	**13/103**	**48/107**	**71/103**	
Crude RR (95% CI)	1.00	3.78 (1.87–8.26)	6.44 (3.24–14.0)	<0.001
p Value	–	<0.001	<0.001	
Adjusted RR (95% CI)[2]	1.00	2.87 (1.02–8.91)	4.09 (1.46–12.9)	0.025
p Value	–	0.045	0.007	
Adjusted RR (95% CI)[3]	1.00	3.85 (1.16–14.7)	5.12 (1.54–20.1)	0.023
p Value	–	0.027	0.007	

[1]Number of subjects for whom information available for all factors included in a log-linear model: 132 HCC cases and 313 controls.
[2]Adjusted for HBV/HCV infection, excluding three HBV+/HCV+ individuals.
[3]Adjusted for HBV/HCV infection, alcohol consumption, smoking habit, BMI 10 years before diagnosis and radiation dose to the liver.

those in the lowest tertile (<0.37 mg/L; heterogeneity $p = 0.32$).

Table 4 shows the association between IL-6 and HCC risk based on tertiles of IL-6. Analyses with adjustment for HBV and HCV infection, alcohol consumption, smoking habit, BMI 10 years before HCC diagnosis and radiation dose showed that RRs of HCC (95% CI) in the middle tertile (2.01–4.46 pg/mL) and highest tertile (>4.46 mg/L) of IL-6

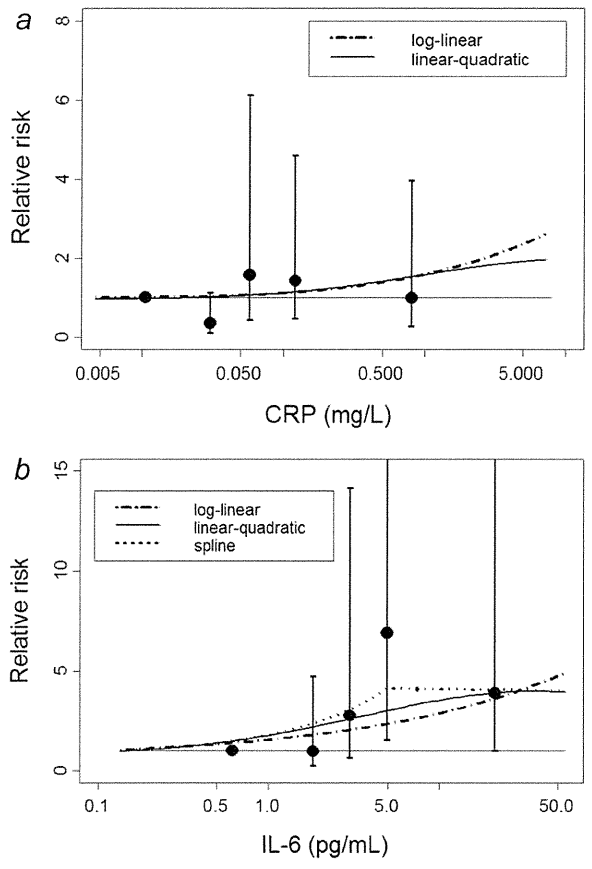

Figure 1. (*a*) Continuous risk of HCC by CRP. RR (95% CI) of HCC with adjustment for alcohol, smoking habit, BMI and radiation dose is plotted according to serum levels of CRP. A test for overall significance of the log-linear curve was not significant (p = 0.23, dashed line). Fit of a linear-quadratic model (solid line) was not as good as the log-linear model according to the AIC model-comparison criterion. (*b*) Continuous risk of HCC by IL-6. RR (95% CI) of HCC with adjustment for alcohol, smoking habit, BMI and radiation dose is plotted according to serum levels of IL-6. A test for overall significance of the log-linear curve was significant (p = 0.015, dashed line). Fits of linear-quadratic (solid line) and linear spline (dotted line) were not as good as the log-linear model according to the AIC model-comparison criterion.

levels were 3.85 (1.16–14.7; p = 0.027) and 5.12 (1.54–20.1; 0.007), respectively, compared to those in the lowest tertile (<2.01 pg/mL; heterogeneity p = 0.023).

Additional analyses were conducted to examine the association between CRP or IL-6 and non-B, non-C HCC risk, although there were relatively few cases with non-B, non-C status (31 cases). Analyses with adjustment for alcohol consumption, smoking habit, BMI 10 years before HCC diagnosis and radiation dose showed that RRs of non-B, non-C HCC (95% CI) in the middle and highest tertiles of CRP were 7.77 (1.13–78.5) and 7.40 (1.26–64.6), respectively, compared to those in the lowest tertile (heterogeneity p = 0.065). RRs of non-B, non-C HCC (95% CI) in the middle and highest tertiles of IL-6 were 56.3 (4.27–2,000) and 98.0 (6.74–4,500), respectively, compared to those in the lowest tertile (heterogeneity p < 0.001) after the same adjustment. The wide confidence bounds are presumably due to the small numbers of non-B, non-C HCC cases.

We also examined the possibility of a nonlinear relation between serum levels of CRP or IL-6 and HCC risk. There was no evidence of any systematic relationship between CRP and HCC risk (Fig. 1*a*). The log RR of HCC increased linearly with logarithm of serum IL-6 level after adjustment for alcohol consumption, smoking habit, BMI and radiation dose (p = 0.015, AIC = 132.63; Fig.1*b*). Although HCC risk appears to level off or decline at high values of IL-6 (Fig.1*b*), neither a negative quadratic term (p = 0.17, AIC = 132.73) nor a linear spline (p = 0.10, AIC = 133.95, with best fit obtained using a join point at log IL-6 = 1.6 or IL-6 = 4.95) revealed any statistically significant departure from the log-linear model. Although the appearance of a downturn at high values of IL-6 may be spurious, lack of statistical significance could also be due to the large uncertainty in estimated risk for IL-6 (high upper bound on confidence intervals for IL-6 groups).

Interaction between IL-6 level and gender, lifestyle-related factors or radiation for risks of HCC

Table 5 shows the association between IL-6 and HCC risk by selected subgroups. Stronger association was found between a 1-SD increase in log IL-6 and HCC risk among subjects with BMI of >25.0 kg/m^2 (obese) 10 years before diagnosis than among subjects with BMI of 21.3–22.9 kg/m^2 (normal), resulting in adjusted RR (95% CI) of 3.09 (1.78–5.81; p for interaction = 0.015). However, there was no significant difference in association between IL-6 and HCC risk among females compared to males, among subjects with alcohol consumption of 40 g of ethanol per day compared to never drinkers, among current smokers compared to never smokers or among subjects exposed to ≥1.0 Gy radiation compared to subjects exposed to <0.001 Gy radiation.

Additional analyses were conducted to examine the association between IL-6 and non-B, non-C HCC risk by selected subgroups. Similarly, a stronger association was found between a 1-SD increase in log IL-6 and non-B, non-C HCC risk among subjects with BMI of >25.0 kg/m^2 than among subjects with BMI of 21.3–22.9 kg/m^2, resulting in adjusted RR (95% CI) of 5.01 (1.51–34.0; p for interaction = 0.025). The results suggest that elevated serum levels of IL-6 among obese subjects are more strongly associated with increased risks of non-B, non-C HCC as well as overall HCC compared to subjects with normal weight.

Discussion

Our study demonstrated that elevated serum levels of IL-6 are associated with increased risk of HCC, independently of hepatitis virus infection, lifestyle-related factors—such as alcohol consumption, smoking habit and BMI—and radiation

Table 5. Relative risks of HCC associated with a 1-SD increase in log IL-6 level

	RR	95% CI	*p* Value for interaction[1]
All HCC	1.84	1.50, 2.28	
Gender			
Males	1.78	1.36, 2.38	
Females	1.91	1.41, 2.68	>0.5
Alcohol consumption (g ethanol per day)			
None	1.91	1.40, 2.69	
≥40	1.88	1.69, 3.53	>0.5
Smoking habit			
Never	2.09	1.48, 3.07	
Current smoker	1.61	1.19, 2.23	0.28
BMI (kg/m^2) 10 years before diagnosis			
21.3–22.9	1.26	0.80, 1.99	
>25.0	3.09	1.78, 5.81	0.015
Radiation dose to the liver (Gy)			
0≤ <0.001	2.01	1.43, 2.89	
≥1.0	2.50	1.38, 5.10	>0.5
Non-B, non-C HCC	1.62	1.14, 2.39	
Gender			
Males	1.09	0.60, 1.96	
Females	2.13	1.32, 3.84	0.09
Alcohol consumption(g ethanol per day)			
None	1.86	1.09, 3.73	
≥40	2.09	0.57, 11.0	>0.5
Smoking habit			
Never	2.04	1.13, 4.16	
Current smoker	1.35	0.78, 2.39	0.33
BMI (kg/m^2) 10 years before diagnosis			
21.3–22.9	0.84	0.31, 2.02	
>25.0	5.01	1.51, 34.0	0.025
Radiation dose to the liver (Gy)			
0≤ <0.001	1.71	0.89, 3.44	
≥1.0	2.66	1.06, 10.1	0.47

[1]*p* Value for interaction is from the likelihood ratio test for a difference in IL-6 risk between high-risk and reference categories of the other factor, while adjustment was made for main effects and interactions of all categories of the other factor.

exposure. Significant association was observed between elevated serum levels of IL-6 and increased risk of non-B, non-C HCC, whereas the association with elevated serum levels of CRP was only marginally significant. Among subjects with obesity, an even stronger association was observed between elevated serum levels of IL-6 and increased risk of HCC (non-B, non-C HCC as well as all HCC).

Several studies have demonstrated that elevated serum level of CRP is associated with poor prognosis in HCC patients, whereas few cohort studies have shown a significant association between CRP level and HCC risk.[39] In our study, the association between serum level of CRP and HCC risk was not significant, after adjusting for HBV and HCV infection, lifestyle-related factors and radiation dose. However, it has been reported that positive association between CRP level and degree of hepatic steatosis occurs among obese patients with nonalcoholic fatty liver disease,[40] and CRP level is useful not only for distinguishing nonalcoholic steatohepatitis (NASH) from simple nonprogressive fatty liver but also for predicting the severity of liver fibrosis in steatohepatitis

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.

Acknowledgements

The authors thank Dr. Kotaro Ozasa for the provision of the tumor registry information, and all members of the Division of Clinical Laboratories for their excellent assistance. The RERF, Hiroshima and Nagasaki, Japan is a private, nonprofit foundation funded by the Japanese Ministry of Health, Labour and Welfare (MHLW) and the U.S. Department of Energy (DOE), the latter in part through DOE Award DE-HS0000031 to the National Academy of Sciences. This publication was supported by RERF Research Protocols #2-75 and #1-09. The views of the authors do not necessarily reflect those of the two governments.

References

1. Umemura T, Kiyosawa K. Epidemiology of hepatocellular carcinoma in Japan. *Hepatol Res* 2007; 37:S95–S100.
2. Abe H, Yoshizawa K, Kitahara T, et al. Etiology of non-B, non-C hepatocellular carcinoma in the eastern district of Tokyo. *J Gastroenterol* 2008;43: 967–74.
3. Gupta K, Krishnaswamy G, Karnad A, et al. Insulin: a novel factor in carcinogenesis. *Am J Med Sci* 2002;323:140–5.
4. El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; 126:460–8.
5. Inoue M, Iwasaki M, Otani T, et al. Diabetes mellitus and the risk of cancer: results from a large-scale population-based cohort study in Japan. *Arch Intern Med* 2006;166:1871–7.
6. Caldwell SH, Crespo DM, Kang HS, et al. Obesity and hepatocellular carcinoma. *Gastroenterology* 2004;127:S97–S103.
7. Marrero JA, Fontana RJ, Fu S, et al. Alcohol, tobacco and obesity are synergistic risk factors for hepatocellular carcinoma. *J Hepatol* 2005;42: 218–24.
8. Saunders D, Seidel D, Allison M, et al. Systematic review: the association between obesity and hepatocellular carcinoma—epidemiological evidence. *Aliment Pharmacol Ther* 2010;31:1051–63.
9. Ioannou GN, Splan MF, Weiss NS, et al. Incidence and predictors of hepatocellular carcinoma in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2007;5:938–45.
10. Calle EE, Rodriguez C, Walker-Thurmond K, et al. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003;348:1625–38.
11. Oh SW, Yoon YS, Shin SA. Effects of excess weight on cancer incidences depending on cancer sites and histologic findings among men: Korea National Health Insurance Corporation Study. *J Clin Oncol* 2005;23:4742–54.
12. Ohishi W, Fujiwara S, Cologne JB, et al. Risk factors for hepatocellular carcinoma in a Japanese population: a nested case-control study. *Cancer Epidemiol Biomarkers Prev* 2008;17:846–54.
13. Jaiswal M, LaRusso NF, Burgart LJ, et al. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Res* 2000;60:184–90.
14. Heikkilä K, Harris R, Lowe G, et al. Associations of circulating C-reactive protein and interleukin-6 with cancer risk: findings from two prospective cohorts and a meta-analysis. *Cancer Causes Control* 2009;20:15–26.
15. Allin KH, Bojesen SE, Nordestgaard BG. Baseline C-reactive protein is associated with incident cancer and survival in patients with cancer. *J Clin Oncol* 2009;27:2217–24.
16. Erlinger TP, Platz EA, Rifai N, et al. C-reactive protein and the risk of incident colorectal cancer. *JAMA* 2004;291:585–90.
17. Pine SR, Mechanic LE, Enewold L, et al. Increased levels of circulating interleukin 6, interleukin 8, C-reactive protein, and risk of lung cancer. *J Natl Cancer Inst* 2011;103:1112–22.
18. Chan AT, Ogino S, Giovannucci EL, et al. Inflammatory markers are associated with risk of colorectal cancer and chemopreventive response to anti-inflammatory drugs. *Gastroenterology* 2011;140:799–808; quiz e11.
19. Zhang SM, Buring JE, Lee IM, et al. C-reactive protein levels are not associated with increased risk for colorectal cancer in women. *Ann Intern Med* 2005;142:425–32.
20. Nakagawa H, Maeda S, Yoshida H, et al. Serum IL-6 levels and the risk for hepatocarcinogenesis in chronic hepatitis C patients: an analysis based on gender differences. *Int J Cancer* 2009;125: 2264–9.
21. Wong VW, Yu J, Cheng AS, et al. High serum interleukin-6 level predicts future hepatocellular carcinoma development in patients with chronic hepatitis B. *Int J Cancer* 2009;124: 2766–70.
22. Naugler WE, Sakurai T, Kim S, et al. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 2007; 317:121–4.
23. Park EJ, Lee JH, Yu GY, et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* 2010;140:197–208.
24. Bromberg J, Wang TC. Inflammation and cancer: IL-6 and STAT3 complete the link. *Cancer Cell* 2009;15:79–80.
25. Bastard JP, Jardel C, Bruckert E, et al. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *Clin Endocrinol Metab* 2000;85:3338–42.
26. Fukuhara T, Sharp GB, Mizuno T, et al. Liver cancer in atomic-bomb survivors: histological characteristics and relationships to radiation and hepatitis B and C viruses. *J Radiat Res* 2001;42: 117–30.
27. Ohishi W, Fujiwara S, Cologne JB, et al. Impact of radiation and hepatitis virus infection on risk of hepatocellular carcinoma. *Hepatology* 2011;53: 1237–45.
28. Cologne JB, Sharp GB, Neriishi K, et al. Improving the efficiency of nested case-control studies of interaction by selecting controls using counter matching on exposure. *Int J Epidemiol* 2004;33: 485–92.
29. Ohishi W, Fujiwara S, Suzuki G, et al. Feasibility of freeze-dried sera for serological and molecular biological detection of hepatitis B and C viruses. *J Clin Microbiol* 2006;44:4593–5.
30. Ohishi W, Fujiwara S, Suzuki G, et al. Validation of the use of freeze-dried sera for the diagnosis of hepatitis B and C virus infections in a longitudinal study cohort. In: Mohan RM, ed. Research advances in microbiology 7. Kerala, India: Global Research Network, 2007. 1–9.
31. Gu Y, Zeleniuch-Jacquotte A, Linkov F, et al. Reproducibility of serum cytokines and growth factors. *Cytokine* 2009;45:44–9.
32. Young RW, Kerr GD, eds. Reassessment of the Atomic Bomb Radiation Dosimetry for Hiroshima and Nagasaki, Dosimetry System 2002, Report of the Joint US-Japan Working Group. Hiroshima, Japan: Radiation Effects Research Foundation, 2005.
33. Sharp GB, Lagarde F, Mizuno T, et al. Relationship of hepatocellular carcinoma to soya food consumption: a cohort-based, case-control study in Japan. *Int J Cancer* 2005;115:290–5.
34. The World Health Organization Western Pacific Region; The International Association for the Study; The International Obesity Task Force. The Asia-Pacific perspective: redefining obesity and its treatment. Sydney, Australia: Health Communications Australia Pty Limited, 2000.
35. Langholz B, Borgan Ø. Counter-matching: a stratified nested case-control sampling method. *Biometrika* 1985;82:69–79.
36. Breslow NE, Day NE. Statistical methods in cancer research, vol. 1—The analysis of case-control studies. Lyon: International Agency for Research on Cancer, 1980.

37. Cologne JB, Tokuoka S, Beebe GW, et al. Effects of radiation on incidence of primary liver cancer among atomic bomb survivors. *Radiat Res* 1999; 153:364–73.
38. Walsh L. A short review of model selection techniques for radiation epidemiology. *Radiat Environ Biophys* 2007;46:205–13.
39. Sieghart W, Pinter M, Hucke F, et al. A single determination of C-reactive protein at the time of diagnosis predicts long term outcome of patients with hepatocellular carcinoma. *Hepatology* 2013; 57:2224–34.
40. Zimmermann E, Anty R, Tordjman J, et al. C-reactive protein levels in relation to various features of non-alcoholic fatty liver disease among obese patients. *J Hepatol* 2011;55: 660–5.
41. Yoneda M, Mawatari H, Fujita K, et al. High-sensitivity C-reactive protein is an independent clinical feature of nonalcoholic steatohepatitis (NASH) and also of the severity of fibrosis in NASH. *J Gastroenterol* 2007;42: 573–82.
42. Wands J. Hepatocellular carcinoma and sex. *N Engl J Med* 2007;357:1974–6.
43. Sander LE, Trautwein C, Liedtke C. Is interleukin-6 a gender-specific risk factor for liver cancer? *Hepatology* 2007;46:1304–5.
44. Prieto J. Inflammation, HCC and sex: IL-6 in the centre of the triangle. *J Hepatol* 2008;48:380–1.
45. Porta C, De Amici M, Quaglini S, et al. Circulating interleukin-6 as a tumor marker for hepatocellular carcinoma. *Ann Oncol* 2008;19:353–8.
46. Grivennikov S, Karin M. Autocrine IL-6 signaling: a key event in tumorigenesis? *Cancer Cell* 2008;13:7–9.
47. Pradhan AD, Manson JE, Rifai N, et al. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 2001;286:327–34.
48. Cologne J, Langholz B. Selecting controls for assessing interaction in nested case-control studies. *J Epidemiol* 2003;13:193–202.

Hepatology Research

Hepatology Research 2013 doi: 10.1111/hepr.12181

Original Article

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

Noriaki Naeshiro,[1] Hideaki Kakizawa,[3] Hiroshi Aikata,[1] Hiromi Kan,[1] Hatsue Fujino,[1] Takayuki Fukuhara,[1] Tomoki Kobayashi,[1] Yohji Honda,[1] Daisuke Miyaki,[1] Tomokazu Kawaoka,[1] Masataka Tsuge,[1] Akira Hiramatsu,[1] Michio Imamura,[1] Yoshiiku Kawakami,[1] Hideyuki Hyogo,[1] Masaki Ishikawa,[2] Kazuo Awai[2] and Kazuaki Chayama[1]

Departments of [1]Gastroenterology and Metabolism, and [2]Diagnostic Radiology, Institute and Graduate School of Biomedical Sciences, Hiroshima University Hospital and [3]Department of Diagnostic Radiology, Hiroshima Red Cross Hospital and Atomic-bomb Survivors Hospital, Hiroshima, Japan

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-

Correspondence: Dr Hiroshi Aikata, Department of Gastroenterology and Metabolism, Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Email: aikata@hiroshima-u.ac.jp

Conflict of interest: The authors have no conflict of interest.

Received 26 April 2013; revision 25 May 2013; accepted 2 June 2013.

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.

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 ($\times 10^4/\mu L$, 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.

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.

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

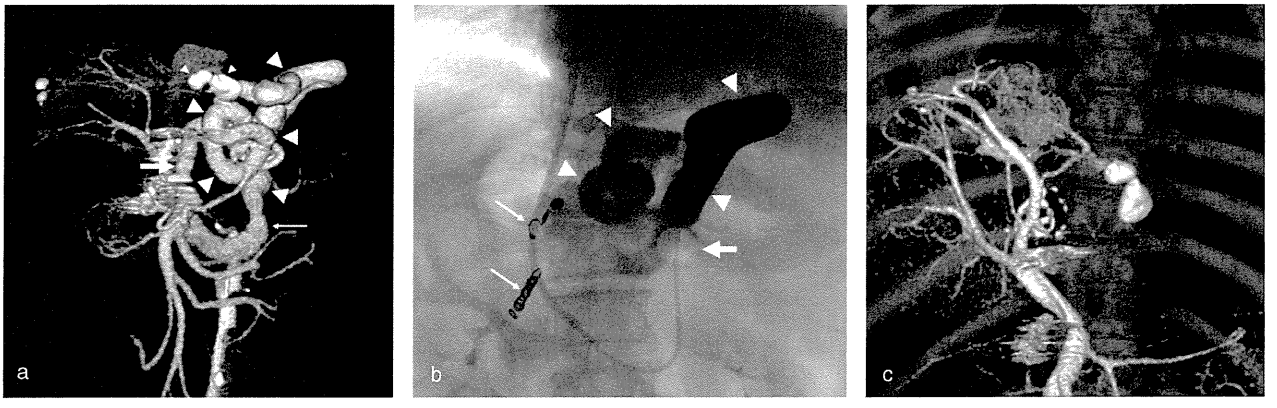

Figure 1 Percutaneous transvenous embolization (PTE) with 5% ethanolamine oleate iopamidol (EOI) for gastrorenal (GR) shunts (patient no. 5). (a) 3-D image obtained by computed tomographic arterial portography via a superior mesenteric artery shows GR shunt (large arrowheads) from a right gastric vein (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.

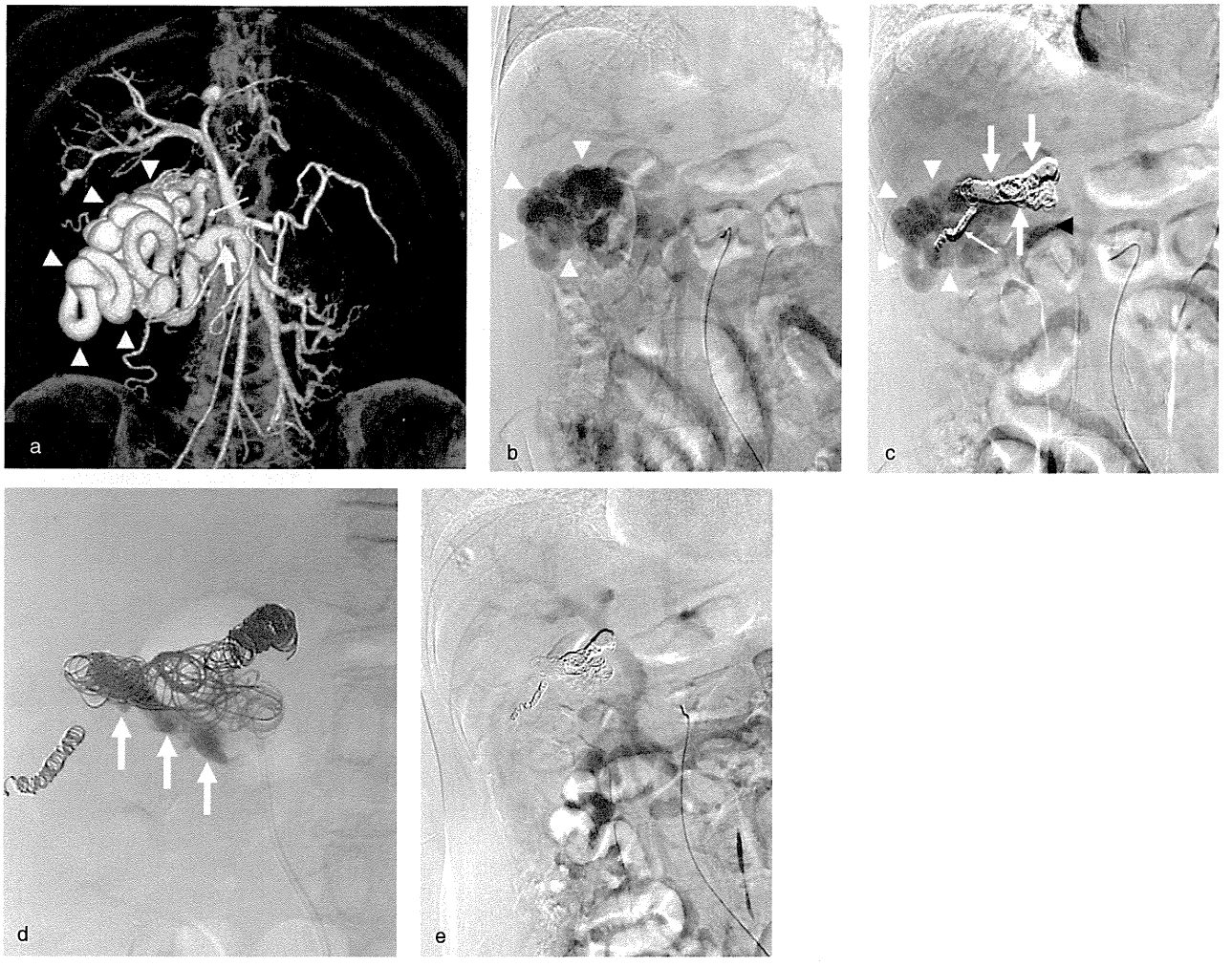

Figure 2 Percutaneous transvenous embolization (PTE) with coils and n-butyl 2-cyanoacrylate (NBCA) for a large superior mesenteric vein (SMV) inferior vena cava (IVC) shunt (patient no. 9). (a) 3-D image obtained by computed tomographic arterial portography via a superior mesenteric artery shows a large 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

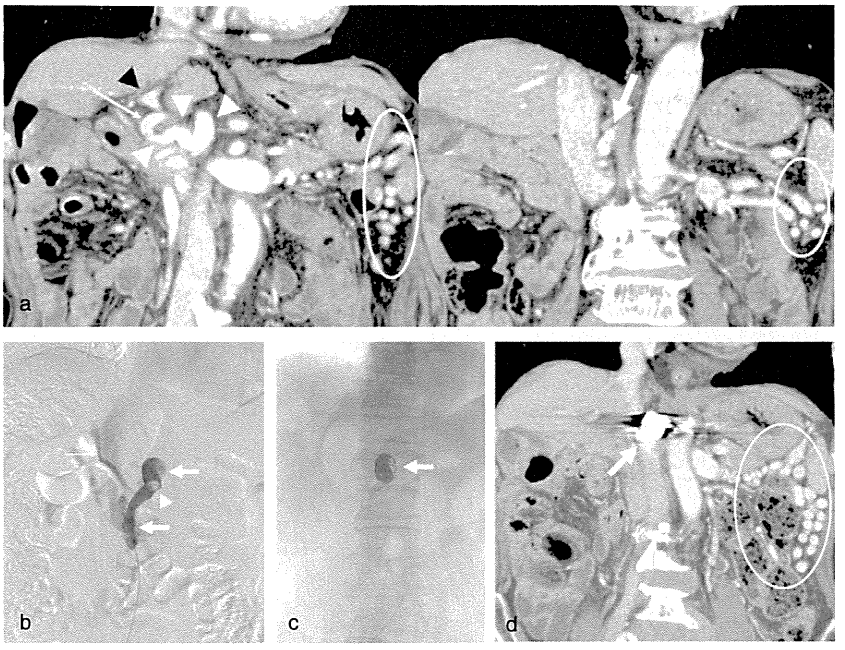

Figure 3 Percutaneous transvenous embolization (PTE) with coils and n-butyl 2-cyanoacrylate (NBCA) for a short portal vein (PV) inferior vena cava (IVC) shunt (patient no. 13). (a) Coronal reconstructed images of intravenous contrast-enhanced computed tomography (CT) shows a short PV-IVC shunt (white 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

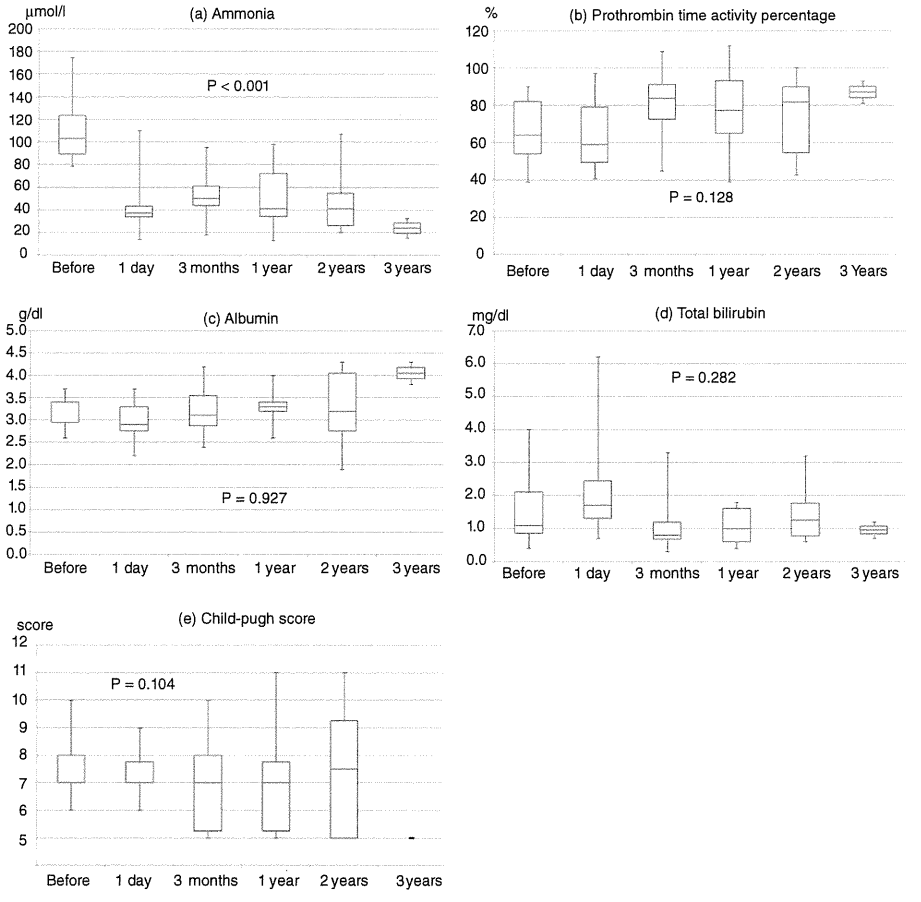

Figure 4 Transitional changes of (a) serum ammonia, (b) prothrombin time activity percentage, (c) albumin, (d) total bilirubin and (e) Child–Pugh score before and after treatment of percutaneous transvenous embolization (PTE). (a) Ammonia level was significantly improved (102 [79–175] vs 41 [13–98] μmol/L; $P < 0.001$) 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.

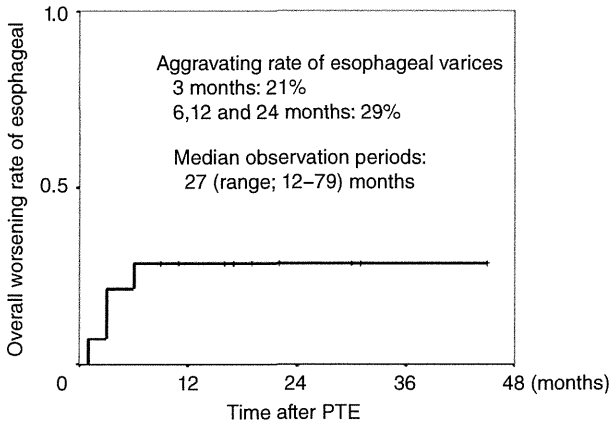

Figure 5 Kaplan–Meier analyses of cumulative aggravated esophageal varices.

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

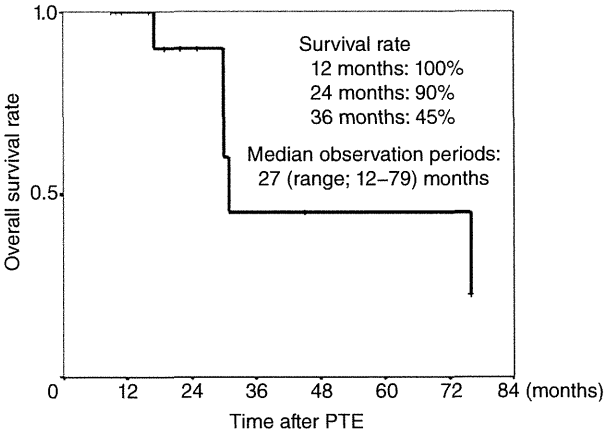

Figure 6 Kaplan–Meier analyses of overall cumulative survival rate. PTE, percutaneous transvenous embolization.

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.

REFERENCES

1 Chung RT, Podolsky DK. Cirrhosis and its complications. In: Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jamesoen JL, eds. *Harrison's Principles of Internal Medicine*, 16th edn. New York: McGraw-Hill, 2005; 1867–9.
2 Sherlock S. *Hepatic Cirrhosis. Diseases of the Liver and Biliary System*, eighth edn. London: Blackwell, 1989; 410–24.
3 Mukund A, Rajesh S, Arora A, Patidar Y, Jain D, Sarin SK. Efficacy of balloon-occluded retrograde transvenous obliteration of large spontaneous lienorenal shunt in patients with severe recurrent hepatic encephalopathy with foam sclerotherapy: initial experience. *J Vasc Interv Radiol* 2012; **23**: 1200–6.
4 Fukuda T, Hirota S, Sugimura K. Long-term results of balloon-occluded retrograde transvenous obliteration for the treatment of gastric varices and hepatic encephalopathy. *J Vasc Interv Radiol* 2001; **12**: 327–36.
5 Kato T, Uematsu T, Nishigaki Y, Sugihara J, Tomita E, Moriwaki H. Therapeutic effect of balloon-occluded retrograde transvenous obliteration on portal-systemic encephalopathy in patients with liver cirrhosis. *Intern Med* 2001; **40**: 688–91.
6 Kawanaka H, Ohta M, Hashizume M *et al.* Portosystemic encephalopathy treated with balloon-occluded retrograde transvenous obliteration. *Am J Gastroenterol* 1995; **90**: 508–10.
7 Yasukawa R, Akiyama F, Tsukishiro T, Narita I. Chronic portal-systemic shunt encephalopathy in a hemodialysis patient treated with balloon-occluded retrograde transvenous obliteration. *Case Rep Nephrol Urol* 2013; **3** (1): 28–33.
8 Tanaka R, Ibukuro K, Abe S *et al.* Treatment of hepatic encephalopathy due to inferior mesenteric vein/inferior vena cava and gonadal vein shunt using dual balloon-