Minor complications and all adverse events that could be regarded as "side effects" were excluded from this analysis.

Information sources

A literature search was conducted on Pubmed and EMBASE to identify clinical series of RFA, PEI and MWA percutaneous procedures for liver tumours published between January 1982 and August 2010. Letters to the editors, supplements, review articles and case reports were excluded and searches were limited to publications of human studies reported in English.

The search strategy formula was peer reviewed by two authors (L.C.B and M.S). A third author (R.T) was consulted for discrepancies.

Study selection

Eligibility assessment of the studies was performed independently in an unblinded way by two reviewers. Disagreements between the reviewers were resolved by consensus.

Data collection process

A data extraction sheet was developed and pilot tested on ten randomly-selected included studies and refined accordingly. One review author extracted the data and a second author checked the extracted data and vice versa. Disagreements were resolved by discussion between the two review authors and if no agreement could be achieved a third author decided.

Country, period of study, year and institution were checked by two authors in order to find duplicate publication and reports.

Data items

Information extracted from each study included: the number of patients, age and Child-Pugh score, first author, country and year of publication.

The type of study were categorized as prospective, retrospective, observational or randomised trial and the type of intervention included radiofrequency ablation, percutaneous ethanol injection and microwave ablation, the tumour number according to type (HCC or metastasis), and the session number for each procedure.

Finally we extracted the data type for outcome measure using number of deaths, major complications and the description of the type of percutaneous ablative technique used and the liver tumour diagnosed.

Assessment of biases

It can be a challenge to assess the possibility of bias in studies reporting treatment complications because they are usually reported as secondary objectives in most trials. Thus we decided to develop a score to evaluate the quality of reports. Selecting a set of criteria to judge the quality of complication reports that were different from those to evaluate the efficacy of treatments [17]. A set of eight criteria were developed incorporating factors potentially associated with more rigorous assessment of adverse events based on which we assigned an overall quality rating to each report. Several of these criteria are similar to those proposed in recent guidelines to improve reporting of complications in randomised trials [18, 19]. Each report was assessed independently by two authors in a blinded manner.

Summary measures and planned statistical analysis

Mortality and complication rates were calculated as a proportion with 95% confidence interval (CI) for each study and then pooled to derive the pooled proportion and 95% CI. The pooled proportion was calculated using Der Simonian-Laird [20] weights for the random effects model in the presence of significant heterogeneity. A

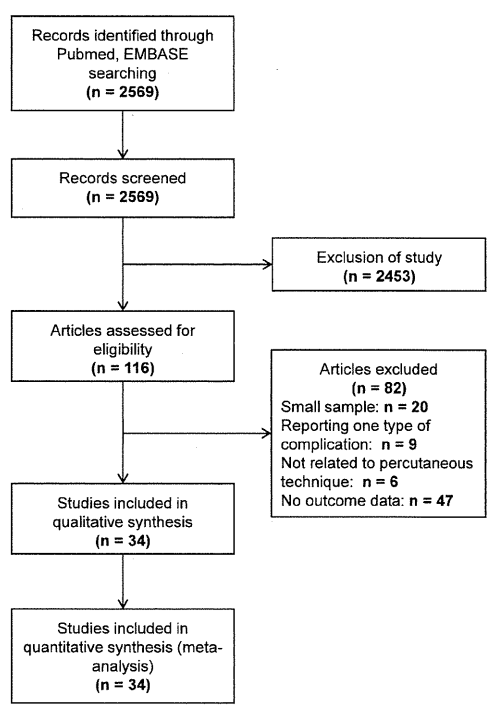

Fig. 1 Study flow diagram

Table 2 Baseline characteristics of the studies included

First author	Country	Year	Patients, n	Age, mean (range)	Child-Pugh class, n			Tumour number		Tumour size, mean (cm)	Intervention	Session number		
					A	B	C	HCC	Metastasis			RFA	PEI	MWA
Randomised	Trials													
Lin [26]	Taiwan	2005	124	NA	93	31	–	142	–	RFA (2.5) PEI (2.3)	RFA/PEI	NA	NA	–
Shibata [27]	Japan	2006	74	65 (41–83)	55	19		83	–	1.9	RFA	95	–	–
Brunello [25]	Italy	2008	139	69	NA	NA	NA	177	–	RFA (2.4) PEI (2.2)	RFA/PEI	NA	NA	–
Observational	Studies													
Shiina [28]	Japan	1993	146	59 (39–82)	77	26	43	242	–	NA	PEI	–	1048	–
Livraghi [29]	Italy	1995	746	64 (31–89)	458	234	41[a]	NA	–	NA	PEI	–	NA	–
Giorgio [30]	Italy	2000	268	63 (42–82)	95	150	23	515	–	5.0	PEI	–	295	–
Livraghi [31]	Italy	2000	114	64 (53–86)	100	14	–	126	–	5.4	RFA	NA	–	–
Ikeda [32]	Japan	2001	119	(23–83)	NA	NA	NA	119	–	RFA (1.8) PEI (1.9)	RFA/PEI	NA	NA	–
Buscarini L [33]	Italy	2001	88	68	56	29	1	101	–	NA	RFA	230	–	–
Livraghi [34]	Italy	2003	2320[b]	NA	NA	NA	NA	NA	–	3.1	RFA	NA	–	–
Guglielmi [35]	Italy	2003	53	68 (48–88)	24	29	–	65	–	4.0	RFA	NA	–	–
Buscarini E [36]	Italy	2003	166	66 (43–75)	79	32	1	147	66	RFA (2.7), PEI (2.4)	RFA/PEI	NA	NA	–
Rhim [37]	Korea	2003	1139	NA	NA	NA	NA	1303	360	NA	RFA	1520	–	–
Ruzzenente [38]	Italy	2004	87	68 (41–88)	48	39	–	104	–	3.9	RFA	130	–	–
Gillams [39]	Italy	2004	167	57 (34–87)	–	–	–	–	685	3.9	RFA	354	–	–
Chen MH [40]	China	2004	110	24–78	26	38	5	74	47	4.7	RFA	536	–	–
Lu DS [41]	US	2005	52	57	19	29	4	87	–	2.5	RFA	76	–	–
Lu MD [42]	China	2005	102	RFA 54 (20–74) MWA 50 (24–74)	69	33	–	170	–	RFA (2.6), MWA (2.5)	RFA/MWA	NA	–	NA
Raut [43]	US,Italy	2005	140	39–86	59	46	35	190	–	3.0	RFA	NA	–	–
Chen [44]	China	2005	338	24–87	96	95	13	430	333	NA	RFA	565	–	–
Cabassa [45]	Italy	2006	59	72 (47–88)	51	8	–	68	–	3.1	RFA	NA	–	–
Solmi [46]	Italy	2006	56	68 (45–81)	16	37	3	63	–	2.8	RFA	68	–	–
Choi [47]	Korea	2007	102	54 (31–73)	77	10[c]	–	119	–	2.0	RFA	107	–	–
Poggi [48]	Italy	2007	250	63	NA	NA	NA	NA	NA	2.9	RFA	292	–	–
Choi D [49]	Korea	2007	570	58	359	160[d]	–	674	–	2.5	RFA	614	–	–
Livraghi [50]	Italy	2008	218	68	NA	NA	NA	218	–	NA	RFA	240	–	–
Kondo [51]	Japan	2008	2480	NA	NA	NA	NA	NA	–	NA	RFA	NA	–	–
Tsai [52]	Taiwan	2008	55	66	39	NA	NA	65	–	2.2	PEI	–	NA	–
Zavaglia [53]	Italy	2008	63	58	46	13	4	71	–	NA	RFA	80	–	–
Chen TM [54]	Taiwan	2008	104	58.6 (28–82)	NA	NA	NA	NA	NA	3.9	RFA	172	–	–
Casaril [55]	United Kingdom	2008	130	65 (33–85)	70	20	2[e]	145	94	2.7	RFA	148	–	–

Table 2 (continued)

First author	Country	Year	Patients, n	Age, mean (range)	Child-Pugh class, n			Tumour number		Tumour size, mean (cm)		Intervention	Session number		
					A	B	C	HCC	Metastasis				RFA	PEI	MWA
Sartori [56]	Italy	2008	181	60 (36–85)	NA	NA	NA	180	181	NA		RFA	NA	–	–
Gillams [57]	United Kingdom	2008	309	64 (24–92)	–	–	–	–	NA		3.7	RFA	617	–	–
Liang [58]	China	2009	1136	54 (23–83)	227	852	57	1385 [f]	516		3.3	MWA		–	3697

[a] 13 cases are unknown

[b] 17 patients had cholangiocellular carcinoma

[c] 8 patients had chronic hepatitis B without cirrhosis, one patient had chronic hepatitis C without cirrhosis, six patients had no evidence of chronic liver disease.

[d] 19 patients had chronic hepatitis B without cirrhosis, 14 patients had chronic hepatitis C without cirrhosis, 18 patients had no evidence of chronic liver disease.

[e] 38 patients with liver metastases

[f] 17 cholangiohepatocellular carcinoma, ten cholangiocarcinoma

NA No data available

random effects model was chosen when the relevant variation in the results was a consequence of several inter-trial differences.

Bias across studies

We checked for the presence of publication bias with Begg's funnel plot, a measure of proportion (on the X axis) against the standard error of proportion (on the Y axis) [21]. We planned a linear regression approach to detect publication bias as described by Egger et al. [22].

Additional analyses

Heterogeneity was tested with the I^2 test, calculating the percentage of variation caused by heterogeneity rather than a chance [23]. It has been suggested that I^2 values of up to 40% might be unimportant, 30% to 60% might be moderate, 50% to 90% may be substantial and 75% to 100% considerable [24].

If significant heterogeneity is found for any outcome, subgroup analysis with the stratification of the variables suspected of causing the inconsistency is recommended. The following dichotomous variables were tested: observational prospective vs. observational retrospective, randomised trials vs. observational, multicenter vs. single centre, RFA vs. PEI vs. MWA, European vs. Asian studies, and good quality vs. fair vs. poor quality studies.

A metaregression analysis was also performed in order to examine the contribution of different variables to the heterogeneity in study results. Covariates selected were: the type of study and number of centres.

We used commercial statistics software (Stats Direct 2.7.7, Stats Direct, Cheshire, United Kingdom) and Comprehensive Metaanalysis V.2 to perform statistical analysis.

Results

Study selection

The search on Medline and EMBASE databases provided a total of 2569 citations (Fig. 1). After screening title and abstract, 2453 were discarded. The full text of the remaining 116 citations was examined in more detail, where 82 studies did not meet the inclusion criteria as described. Finally 34 publications were included in the metaanalysis.

Study characteristics

Study design, participants and interventions

Of the 34 studies finally selected for the review, three were randomised trials [25–27] and 31 were observational studies (Table 2) [28–58]. Eighteen studies were from Europe, fourteen studies from Asia and one study from the United States. There was one study conducted in collaboration between United States and Italy. Most of the reports were published after 2000 (n=32).

There were 24 studies using RFA only, four assessing PEI as only treatment and one using MWA only. Two randomised control trials and two observational studies compared RFA and PEI. One observational study evaluated RFA versus MWA. The included studies involved 12 158 participants. According to the type of technique, 9531, 1185 and 1442 patients were included for RFA, MWA and PEI, respectively. The average age of patients ranged from 24 to 89 years. Mean tumour size treated ranged from 1.8 to 5.0 cm.

Outcomes

In 13 studies, mortality and complications were weighted as primary outcomes. Death and adverse events were assessed as secondary outcomes in 21 studies. Mean follow-up after treatment ranged from 10.3 to 134 months.

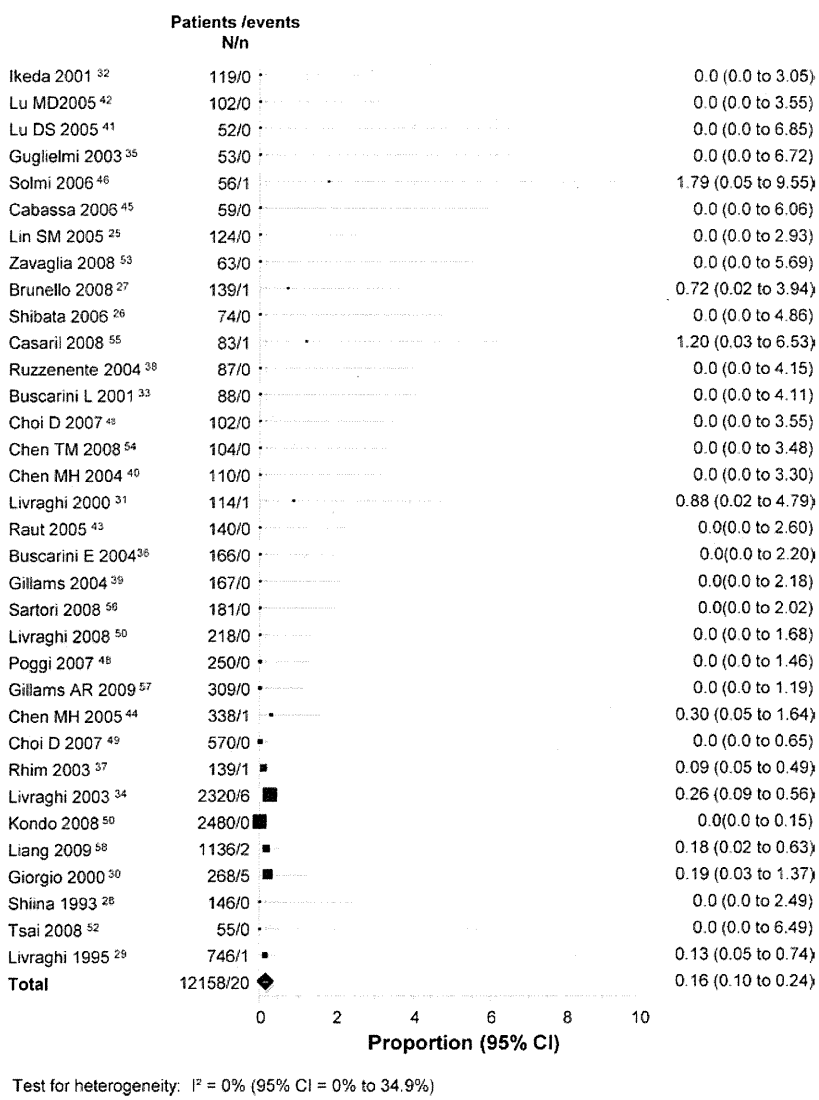

Fig. 2 Forest plot of overall mortality rates of ablative techniques

Quality of studies

According to the quality rating criteria, there were 8, 23 and 3 articles assessed as, "good ", "satisfactory" and "poor" respectively. The eight good articles included one randomised control trial, three observational prospective and four observational retrospective studies.

Results of individual studies

Primary outcome

Mortality was reported in all studies. For all percutaneous ablative techniques analysed, mortality ranged from 0% to 0.88% and the pooled proportion was 0.16% (95%CI, 0.10–0.24%) by the random effects model. There was no evidence of heterogeneity (I^2=0%; 95% CI, 0–35%; Fig. 2).

Individual analysis for each technique showed a pooled mortality of 0.15% (95% CI, 0.08%–0.23%) for RFA, 0.59% (95% CI, 0.14%–1.3%) for PEI and 0.23% (95% CI, 0.0%–0.58%) for MWA. No evidence of significant heterogeneity was detected (Fig. 3). Beggs funnel plot and Egger's test (0.18, P=0.06) showed no evidence of publication bias.

Complications related to the death are summarised in Table 3. According to the type of technique there were 11, 7 and two deaths related to RFA, PEI and MWA respectively.

Eighteen cases involved HCC and two cases liver metastasis.

Secondary outcome

Complication rates by proportion based on the random effects model for the three percutaneous ablative techniques analyzed ranged from 0% to 17%, and the pooled proportion was 3.29% (95% CI, 2.43%–4.28%). Significant heterogeneity was found for this outcome (I^2=84.8%, 95% CI 79.9–88%). Begg's funnel plot and Egger's test (1.57, P=0.11) showed no evidence of publication bias.

Table 4 summarised the ten most frequent major complications found on the review classified according to the types of technique and tumour. Other major complica-

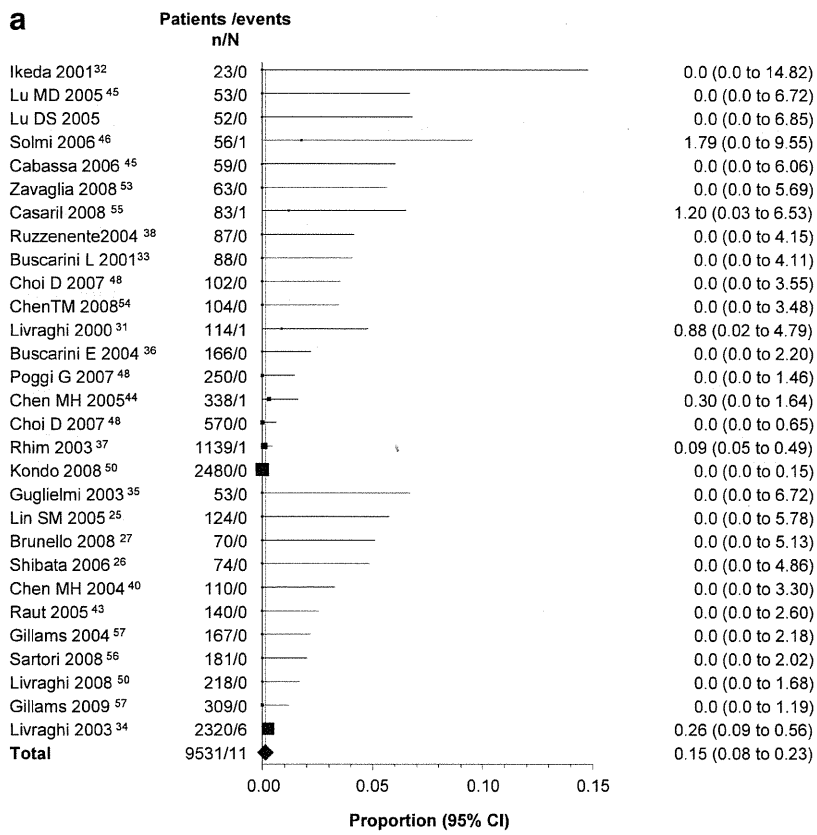

Fig. 3 Forest plots of radiofrequency ablation (RFA) (**a**), percutaneous ethanol injection (PEI) (**b**) and microwave ablation (MWA) (**c**) mortality rates

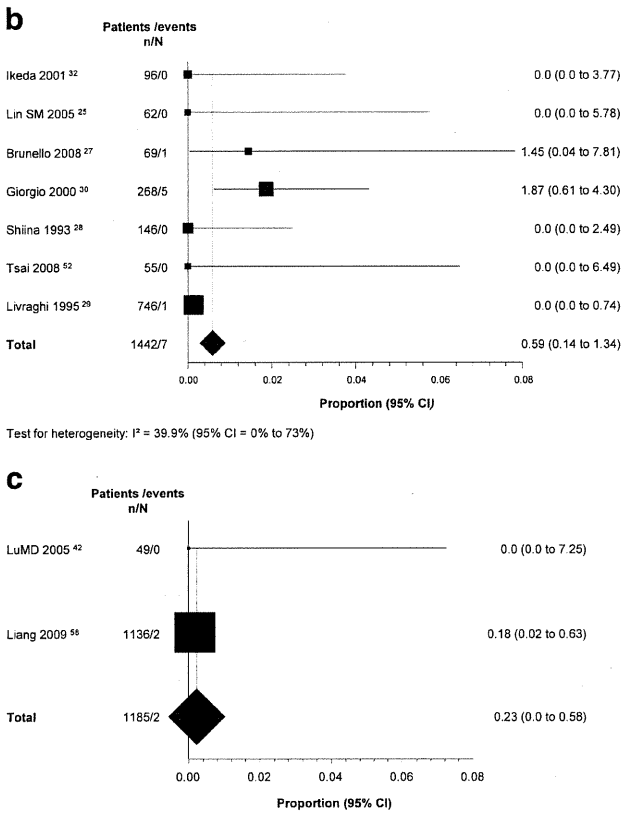

Fig. 3 (continued)

tions reported included pneumothorax (n=8), acute cholecystitis (n=6), biloma (n=9), rapid tumour progression (n=4), chest infection (n=3), visceral injury (n=3), colon perforation (n=3), subcapsular haematoma (n=2), bile duct injury (n=2), portal thrombosis (n=2), arrhythmias (n=2), bacterial endocarditis (n=2), arteriovenous fistula (n=2) and intrapleural bleeding (n=2) for radiofrequency ablation.

Major complications also included acute tubular necrosis (n=2), pleurisy (n=2) haemobilia (n=2), acute cholangitis (n=2) for PEI, and skin burn requiring resection (n=3), colon perforation (n=2) and subcapsular haematoma (n=2) for MWA.

Additional analysis

As the 34 studies showed a significant heterogeneity for the secondary outcome, a subgroup analysis according to the type of study (observational prospective and retrospective vs. randomised trials, number of centres (single vs. multicenter), type of technique (RFA, PEI and MWA) and ethnic origin of patients (European vs. Asian) were performed as planned (Table 5). Consistent results were observed only when assessing data pooled from randomised trials and Asian studies. Moderate heterogeneity was found when assessing data pooled from multicenter studies.

Table 6 (Appendix) shows subgroup analysis with the pooled data of studies grouped based on quality rating (poor, satisfactory/fair, or good). Higher complication rates were associated with reports with complications as primary outcome, prospective studies with early and late complications defined, those without information on outcome of more than half cases of complication and those with no definition of major complications. Single-centre and prospective studies reported higher complication rates than did multicentre and retrospective studies.

Meta-regression models with covariates for the secondary outcome and restricted to radiofrequency ablation data of complications showed a significant statistical difference between prospective and retrospective studies (P<0.001) as well between multicentre and single-centre studies (P<0.001) (Appendix Fig. 4). Howev-

Table 3 Summary of complications related to death according to type of technique and tumour

Complications related to death	Number	Type of technique	Type of tumour	Reference
Cardiac failure	1	MWA	HCC	[58]
Multiorgan failure	1	MWA	HCC	[58]
Colon perforation	3	RFA	HCC	[34, 55]
Peritonitis	2	RFA	HCC	[31, 34]
Liver failure	3	RFA(2),PEI(1)	HCC	[30, 34]
Tumour rupture with massive haemorrhage	1	RFA	HCC	[34]
Sudden death	1	RFA	Metastasis	[34]
Peritoneal haemorrhage	2	RFA(1) PEI(1)	HCC	[30]
Portal vein thrombosis	1	PEI	HCC	[25]
Oesophageal variceal bleeding	4	PEI	HCC	[29, 30]
Bile leakage	1	RFA	Metastasis	[44]

Table 4 Summary of the ten most frequent major complications of ablative techniques found in the review*

Major complications by type	Number	%	Type of technique	Type of tumour	Reference
Tumour seeding	61	0.50	MWA (5), RFA (56)	HCC (23),Metastasis (6), NA (32)	[27, 34, 38, 40, 49–51, 55, 56, 58],,.
Intraperitoneal haemorrhage	47	0.37	RFA (37), PEI (10)	HCC (33), Metastasis (4), NA (10)	[28–31, 34, 36–38, 44–47, 50, 53, 54, 56]
Liver abscess	39	0.32	MWA (4), RFA (31) PEI (4)	HCC (15), Metastasis (4), NA (20)	[29, 30, 34, 37, 44, 48–50, 54, 58]
Ascites	34	0.27	PEI (33), MWA (1)	HCC	[30]
Pleural effusion requiring treatment	18	0.14	MWA (13), RFA(5)	HCC (8), Metastasis (8), NA (2)	[28, 43, 46, 50, 52, 58]
Hepatic infarction	16	0.13	RFA(15), PEI(1)	HCC (7), NA (9)	[29, 34, 37, 48–50]
Liver failure	13	0.11	RFA(5), PEI(8)	HCC (12), NA (1)	[30, 34, 37]
Perforation of the gastrointestinal wall [1]	13	0.11	RFA	Metastasis (4), NA (9)	[34, 50]
Haemothorax	11	0.09	RFA	HCC (7), Metastasis (3), NA (1)	[25, 26, 34, 37, 44, 47, 50]
Haemorrhage requiring Treatment[2]	11	0.09	RFA	HCC (3), Metastasis (8)	[39, 41, 44]

*Numbers in parentheses indicate the number of complications according to the type of technique

NA Data not available

[1] 3 patients with colon perforation

1 patient with stomach perforation

9 patients with no available information about location

[2] 8 patients with hemorrhage requiring transfusion

1 patient with arterial hemorrhage requiring embolization

2 Intraperitoneal hemorrhage requiring ultrasound guide ablation

Table 5 Heterogeneity analysis with stratifying variables for major complications

	Number of studies	Pooled proportion (%)	95% CI	I^2, (%)
All studies	34	3.2	2.4–4.3	84
Study type				
Observational prospective	13	5.3	3.4–7.6	68
Observational retrospective	18	3.8	2.7–4.9	85
Randomized trials	3	2.5	1.0–4.3	0
Number of centres				
Multicenter	6	2.5	1.4–4.4	44
Single centre	28	4.5	3.3–6.0	84
Ethnic origin of patients				
Asia	14	3.4	2.6–4.3	34
Europe	18	4.1	2.5–6.5	47
Type of technique				
RFA	29	4.1	3.3–5.1	71
PEI	7	2.7	0.28–7.4	93
MWA	2	4.6	0.7–11.8	–

er these two metaregression models do not explain completely the heterogeneity among trials.

Discussion

In this systematic review and meta-analysis, mortality and major complication rates for the three percutaneous ablative techniques included were 0.16% and 3.29% respectively. The results without heterogeneity show a mortality of 0.15%, 0.59% and 0.23% for RFA, PEI and MWA, respectively. Reported major complication rates, calculated by using a random effects model in the presence of significant heterogeneity, were 4.1% for RFA, 2.7% for PEI and 4.6% for MWA. Significant heterogeneity was found in complication rates, except in the subgroup of randomised trials and of Asian studies probably because most of the studies were observational with wide differences in populations, design and inclusion criteria.

The results from this metaanalysis supported previous reports of randomised control trials [25, 26] assessing

efficacy and safety of RFA and PEI, showing that both techniques can be considered safe in terms of mortality and complication rates.

Bouza et al. conducted a metaanalysis on six randomised trials evaluating the efficacy and safety of RFA and PEI including major complications. In this study major complication rates for RFA and PEI were 4.2% and 2.7%, respectively, which were similar to our present results. No significant statistical difference was found between the two treatments [59].

Microwave ablation-associated mortality was reported to occur in 0.002% according to a systematic review of this technique [60]. Major complication rates have been reported to be higher with MWA than with RFA in a randomised trial [61]. Our results indicated that MWA is a safe technique in terms of mortality and major complication rate. However the results should be interpreted with caution because it was based on one large study. More reports including large number of patients are needed to make a solid conclusion.

The major complication rates reported differed between single-/multicentre studies and prospective/retrospective studies. Single-centre studies may report higher complication rates compared with multicentre studies because of a real-time evaluation of complications rather than the questionnaire-dependent data collection in multicentre studies. Prospective studies reported higher complication rates compared with retrospective studies. This result may be explained by a number of different factors; prospective studies may report more accurately the number of participants lost to follow-up, the timing of collecting complications and the adequate predefined definitions for harms.

Neoplastic seeding is a well-recognised complication of RFA. A small-scale study published 10 years ago suggested a risk of as high as 12.5% [62]. A more recent large scale multicentre study on the other hand identified a lower risk of 0.9% [63]. A recent systematic review highlighted an overall median risk of 0.6%; however it reported a higher risk of tumour seeding in HCC when combining percutaneous radiofrequency ablation and liver biopsy [64]. We report tumour seeding in 61 patients and most of the cases involved HCC. Estimation of the incidence for this complication based on these data may not be accurate because of incomplete reports and inadequate length of follow-up. Incomplete reports may include cases of tumour seeding solved by treatment. Length of follow-up differs from study to study and can affect the incidence of tumour seeding reported. Also the rate of neoplastic seeding may be affected by other factors such as technical issues and subcapsular location or histological degree/differentiation. Appropriate measures can reduce the risk of tumour seeding as suggested by many authors [65–67].

The metaanalysis reported here has several limitations. The combined data from randomised and observational studies may represent a source of important bias, the quality of the studies varied and the quality rating criteria tools used have not been validated previously. Even with the advantage of including only studies reporting major complications, controversies exist among authors, and terminology for the appropriate classification of major complications have not been widely used. All of these may have led to significant heterogeneity for the second outcome.

Others limitation of this study are restriction to English language publications and the poor or incomplete reporting concerning adequate classification of major complications. All future trials should include standard definitions for major complications as proposed in the guidelines for image-guided tumour ablation [15]. Publication bias is a concern for all systematic reviews. Poor-quality assessment of harms in observational studies or the fact that observational studies are more likely to be published if they report good results represents another drawback of this metaanalysis as most of the reports included were observational studies. However by including only randomised controlled trials, useful information concerning harms may be lost. Randomised controlled trials prespecified hypotheses for adverse events and assess homogeneous population and settings. Patients who are more susceptible to adverse events are often underrepresented in such efficacy trials; thus observational studies may provide the only evidence for evaluating harms in minority or vulnerable populations.

In this study, some subgroup analyses including a comparison between HCC patients with cirrhosis and patients with liver metastases could not be computed because some reports did not describe whether patients with complications had HCC or metastatic liver tumor. Patients with cirrhosis are prone to higher rate of complications because of tendency to bleeding, ascites or liver failure.

In conclusion mortality and major complication rates associated with percutaneous ablation were acceptably low for the three techniques analysed. Future reviews should evaluate the presence of rare adverse events by including case reports, the influence of the number and type of tumours and the pooled estimate of risks stratified by study design.

Acknowledgement This work was supported in part by Health Sciences Research Grants of The Ministry of Health, Labour and Welfare of Japan (Research on Hepatitis).The supporters had no role in study design; collection, analysis, interpretation of data; writing of the report or decision to submit the manuscript for publication.

Appendix

Table 6 Quality assessment tool for studies reporting complications of ablative techniques for the treatment of liver tumors

Criteria	Score	Studies, n	Pooled proportion major complications 95% CI	I^2 (%)
1 .Adequate report of complications without missing	1i 1: Complications were prospectively observed	15	4.8 (3.1–7.4)	54
	0: Only retrospective observation	19	3.5 (2.5–4.9)	89
	1i 1: Complications rate is reported from single center	28	4.4 (3.4–5.8)	44
	0: Complications rate is reported from multicenter.	6	2.9(1.7–4.5)	84
2. Definition of complications	1: Study reports explicit definitions for major complications allow for SIR classification.	13	3.6 (2.3–5.7)	86
	0: Without any definition of major complications or without adequate basis of classification	21	4.1(2.9–5.9)	83
3.Weight of complication as an endpoint	1: complication is one of the primary outcomes	13	4.7(3.0–7.2)	87
	0: complication is just a secondary outcome	21	3.3(2.2–5.0)	84
4. Adequate stratification of patients	1: proportion of patients with each Child-Pugh class is reported.	26	4.0 (2.8–5.7)	87
	0: without any information of Child-Pugh class of each patient.	6	3.2 (1.6–6.3)	67
5. Complication period	1: early and late complications were distinguished.	6	4.7 (2.4–9.3)	85
	0: no mention of period	28	3.7 (2.7–5.1)	86
6. Course of complications	1: the course or treatment of more than half of major complication is reported	24	3.7(2.6–5.2)	86
	0: more than half of major complications are without any information of their course.	10	4.5(2.6–7.6)	84
7. Sessions	1: the session number of each procedure is reported	24	3.9 (2.7–5.5)	78
	0: without any information of session number	10	3.7(2.2–6.5)	87
8. Adequate duration of follow-up:	1: Study reports duration of follow-up and duration of follow-up is adequate to identify expected adverse events (at least 1 year for studies of ablative techniques)	32	3.9 (2.8–5.2)	86
	0: No report of follow up or inadequate.	2	4.4(1.5–12.0)	67
Total score (0–9)	>6: Good	8	4.2 (2.3–7.5)	80
	4–6: Satisfactory fair	23	4.1 (2.8–5.8)	86
	<4: Poor	3	3.7 (1.1–6.1)	62

SIR Society of interventional radiology

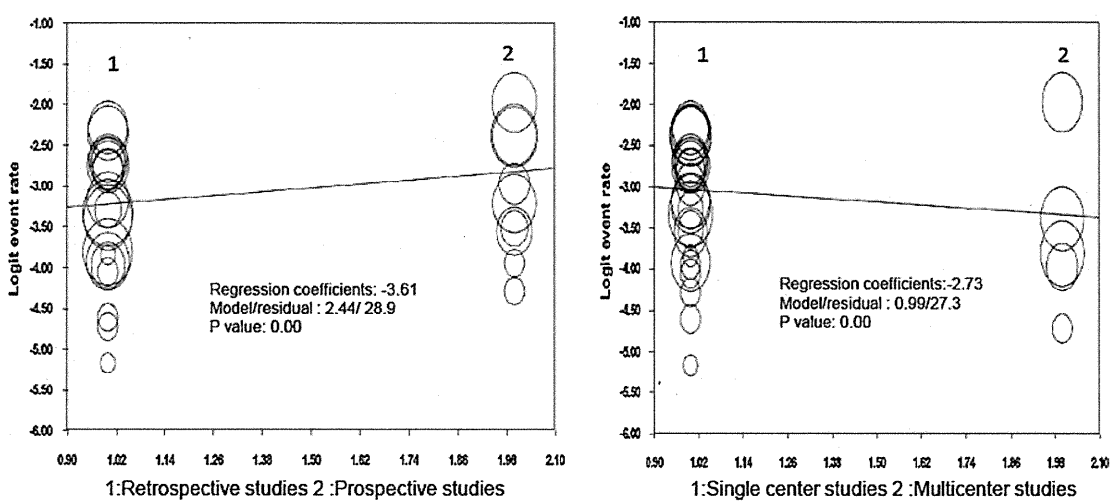

Fig. 4 Metaregression models with covariates restricted to major complications of radiofrequency ablation

References

1. Sutherland LM, Williams JA, Padbury RT, Gotley DC, Stokes B, Maddern GJ (2006) Radiofrequency ablation of liver tumors: a systematic review. Arch Surg 141:181–190
2. Sherman M (2010) Hepatocellular carcinoma: epidemiology, surveillance, and diagnosis. Semin Liver Dis 30:3–16
3. Yuen MF, Hou JL, Chutaputti A (2009) Hepatocellular carcinoma in the Asia pacific region. J Gastroenterol Hepatol 24:346–353
4. Vanagas T, Gulbinas A, Pundzius J, Barauskas G (2010) Radiofrequency ablation of liver tumors (I): biological background. Medicina 46:13–17
5. Clinical Practice Guidelines for Hepatocellular Carcinoma—The Japan Society of Hepatology 2009 update. Hepatol Res 40(Suppl 1):2–144
6. Bruix J, Sherman M (2010) Management of hepatocellular carcinoma: an update. Hepatology (American Association for Study of Liver Disease website. Available at http://www.aasld.org/practiceguidelines/Documents/Bookmarked%20Practice%20Guidelines/HCCUpdate2010.pdf (Accessed December 28 2010))
7. Omata M, Lesmana LA, Tateishi R et al (2010) Asian Pacific Association for the Study of the liver consensus recommendations on hepatocellular carcinoma. Hepatol Int 4:439–474
8. Lencioni R (2010) Loco-regional treatment of hepatocellular carcinoma. Hepatology 52:762–773
9. Lencioni R, Crocetti L, De Simone P, Filipponi F (2010) Loco-regional interventional treatment of hepatocellular carcinoma: techniques, outcomes, and future prospects. Transpl Int 23:698–703
10. Curley SA, Marra P, Beaty K et al (2004) Early and late complications after radiofrequency ablation of malignant liver tumors in 608 patients. Ann Surg 239:450–458
11. de Baere T, Risse O, Kuoch V et al (2003) Adverse events during radiofrequency treatment of 582 hepatic tumors. AJR Am J Roentgenol 181:695–700
12. Kasugai H, Osaki Y, Oka H, Kudo M, Seki T (2007) Severe complications of radiofrequency ablation therapy for hepatocellular carcinoma: an analysis of 3,891 ablations in 2,614 patients. Oncology 72(Suppl 1):72–75
13. Tateishi R, Shiina S, Teratani T et al (2005) Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. Cancer 103:1201–1209
14. Crocetti L, de Baere T, Lencioni R (2010) Quality improvement guidelines for radiofrequency ablation of liver tumours. Cardiovasc Intervent Radiol 33:11–17
15. Goldberg SN, Grassi CJ, Cardella JF et al (2005) Image-guided tumor ablation: standardization of terminology and reporting criteria. Radiology 235:728–739
16. Omary RA, Bettmann MA, Cardella JF et al (2003) Quality improvement guidelines for the reporting and archiving of interventional radiology procedures. J Vasc Interv Radiol 14: S293–S295
17. Chou R, Helfand M (2005) Challenges in systematic reviews that assess treatment harms. Ann Intern Med 142:1090–1099
18. Ioannidis JPA, Evans SJW, Gotzsche PC et al (2004) Better reporting of harms in randomized trials: an extension of the CONSORT statement. Ann Intern Med 141:781–788
19. Loke YK, Price D, Herxheimer A, Caem G (2007) Systematic reviews of adverse effects: framework for a structured approach. BMC Med Res Methodol 7:32
20. Dersimonian R, Laird N (1986) Metaanalysis in clinical-trials. Control Clin Trials 7:177–188
21. Begg CB, Mazumdar M (1994) Operating characteristics of a rank correlation test for publication bias. Biometrics 50(4):1088–1101
22. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315:629–634
23. Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327:557–560
24. Higgins JPT GS Cochrane handbook for systematic reviews of interventions. Version 5.0.0 [updated February 2008]: The Cochrane Collaboration; . Available from: www.cochrane-handbook.org (Accessed August 28 2010)
25. Brunello F, Veltri A, Carucci P et al (2008) Radiofrequency ablation versus ethanol injection for early hepatocellular carcinoma: a randomized controlled trial. Scand J Gastroenterol 43:727–735
26. Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC (2005) Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. Gut 54:1151–1156
27. Shibata T, Maetani Y, Isoda H, Hiraoka M (2006) Radiofrequency ablation for small hepatocellular carcinoma: prospective comparison of internally cooled electrode and expandable electrode. Radiology 238:346–353
28. Shiina S, Tagawa K, Niwa Y et al (1993) Percutaneous ethanol injection therapy for hepatocellular carcinoma: results in 146 patients. AJR Am J Roentgenol 160:1023–1028
29. Livraghi T, Giorgio A, Marin G et al (1995) Hepatocellular carcinoma and cirrhosis in 746 patients: long-term results of percutaneous ethanol injection. Radiology 197:101–108
30. Giorgio A, Tarantino L, de Stefano G et al (2000) Ultrasound-guided percutaneous ethanol injection under general anesthesia for the treatment of hepatocellular carcinoma on cirrhosis: long-term results in 268 patients. Eur J Ultrasound 12:145–154
31. Livraghi T, Goldberg SN, Lazzaroni S et al (2000) Hepatocellular carcinoma: radio-frequency ablation of medium and large lesions. Radiology 214:761–768
32. Ikeda M, Okada S, Ueno H, Okusaka T, Kuriyama H (2001) Radiofrequency ablation and percutaneous ethanol injection in patients with small hepatocellular carcinoma: a comparative study. Jpn J Clin Oncol 31:322–326
33. Buscarini L, Buscarini E, Di Stasi M, Vallisa D, Quaretti P, Rocca A (2001) Percutaneous radiofrequency ablation of small hepatocellular carcinoma: long-term results. Eur Radiol 11:914–921
34. Livraghi T, Solbiati L, Meloni MF et al (2003) Treatment of focal liver tumors with percutaneous radio-frequency ablation: complications encountered in a multicenter study. Radiology 226:441–451
35. Guglielmi A, Ruzzenente A, Battocchia A, Tonon A, Fracastoro G, Cordiano C (2003) Radiofrequency ablation of hepatocellular carcinoma in cirrhotic patients. Hepatogastroenterology 50:480–484
36. Buscarini E, Buscarini L (2004) Radiofrequency thermal ablation with expandable needle of focal liver malignancies: complication report. Eur Radiol 14:31–37
37. Rhim H, Yoon KH, Lee JM et al (2003) Major complications after radio-frequency thermal ablation of hepatic tumors: spectrum of imaging findings. Radiographics 23:123–134
38. Ruzzenente A, Manzoni GD, Molfetta M et al (2004) Rapid progression of hepatocellular carcinoma after radiofrequency ablation. World J Gastroenterol 10:1137–1140
39. Gillams AR, Lees WR (2004) Radio-frequency ablation of colorectal liver metastases in 167 patients. Eur Radiol 14:2261–2267
40. Chen MH, Yang W, Yan K et al (2004) Large liver tumors: protocol for radiofrequency ablation and its clinical application in 110 patients–mathematic model, overlapping mode, and electrode placement process. Radiology 232:260–271

41. Lu DS, Yu NC, Raman SS et al (2005) Percutaneous radiofrequency ablation of hepatocellular carcinoma as a bridge to liver transplantation. Hepatology 41:1130–1137
42. Lu MD, Xu HX, Xie XY et al (2005) Percutaneous microwave and radiofrequency ablation for hepatocellular carcinoma: a retrospective comparative study. J Gastroenterol 40:1054–1060
43. Raut CP, Izzo F, Marra P et al (2005) Significant long-term survival after radiofrequency ablation of unresectable hepatocellular carcinoma in patients with cirrhosis. Ann Surg Oncol 12:616–628
44. Chen MH, Yang W, Yan K et al (2005) Treatment efficacy of radiofrequency ablation of 338 patients with hepatic malignant tumor and the relevant complications. World J Gastroenterol 11:6395–6401
45. Cabassa P, Donato F, Simeone F, Grazioli L, Romanini L (2006) Radiofrequency ablation of hepatocellular carcinoma: long-term experience with expandable needle electrodes. AJR Am J Roentgenol 186:S316–S321
46. Solmi L, Nigro G, Roda E (2006) Therapeutic effectiveness of echo-guided percutaneous radiofrequency ablation therapy with a LeVeen needle electrode in hepatocellular carcinoma. World J Gastroenterol 12:1098–1104
47. Choi D, Lim HK, Rhim H et al (2007) Percutaneous radiofrequency ablation for early-stage hepatocellular carcinoma as a first-line treatment: long-term results and prognostic factors in a large single-institution series. Eur Radiol 17:684–692
48. Choi D, Lim HK, Rhim H et al (2007) Percutaneous radiofrequency ablation for recurrent hepatocellular carcinoma after hepatectomy: long-term results and prognostic factors. Ann Surg Oncol 14:2319–2329
49. Poggi G, Riccardi A, Quaretti P et al (2007) Complications of percutaneous radiofrequency thermal ablation of primary and secondary lesions of the liver. Anticancer Res 27:2911–2916
50. Livraghi T, Meloni F, Di Stasi M et al (2008) Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: is resection still the treatment of choice? Hepatology 47:82–89
51. Kondo Y, Yoshida H, Tateishi R, Shiina S, Kawabe T, Omata M (2008) Percutaneous radiofrequency ablation of liver cancer in the hepatic dome using the intrapleural fluid infusion technique. Br J Surg 95:996–1004
52. Tsai WL, Cheng JS, Lai KH et al (2008) Clinical trial: percutaneous acetic acid injection vs. percutaneous ethanol injection for small hepatocellular carcinoma—a long-term follow-up study. Aliment Pharm Therapy 28:304–311
53. Zavaglia C, Corso R, Rampoldi A et al (2008) Is percutaneous radiofrequency thermal ablation of hepatocellular carcinoma a safe procedure? Eur J Gastroenterol Hepatol 20:196–201
54. Chen TM, Huang PT, Lin LF, Tung JN (2008) Major complications of ultrasound-guided percutaneous radiofrequency ablations for liver malignancies: single center experience. J Gastroenterol Hepatol 23:e445–e450
55. Casaril A, Abu Hilal M, Harb A, Campagnaro T, Mansueto G, Nicoli N (2008) The safety of radiofrequency thermal ablation in the treatment of liver malignancies. Eur J Surg Oncol 34:668–672
56. Sartori S, Tombesi P, Macario F et al (2008) Subcapsular liver tumors treated with percutaneous radiofrequency ablation: a prospective comparison with nonsubcapsular liver tumors for safety and effectiveness. Radiology 248:670–679
57. Gillams AR, Lees WR (2009) Five-year survival in 309 patients with colorectal liver metastases treated with radiofrequency ablation. Eur Radiol 19:1206–1213
58. Liang P, Wang Y, Yu X, Dong B (2009) Malignant liver tumors: treatment with percutaneous microwave ablation–complications among cohort of 1136 patients. Radiology 251:933–940
59. Bouza C, Lopez-Cuadrado T, Alcazar R, Saz-Parkinson Z, Amate JM (2009) Meta-analysis of percutaneous radiofrequency ablation versus ethanol injection in hepatocellular carcinoma. BMC Gastroenterol 9:31
60. Ong SL, Gravante G, Metcalfe MS, Strickland AD, Dennison AR, Lloyd DM (2009) Efficacy and safety of microwave ablation for primary and secondary liver malignancies: a systematic review. Eur J Gastroenterol Hepatol 21:599–605
61. Shibata T, Iimuro Y, Yamamoto Y et al (2002) Small hepatocellular carcinoma: comparison of radio-frequency ablation and percutaneous microwave coagulation therapy. Radiology 223:331–337
62. Llovet JM, Vilana R, Bru C et al (2001) Increased risk of tumor seeding after percutaneous radiofrequency ablation for single hepatocellular carcinoma. Hepatology 33:1124–1129
63. Livraghi T, Lazzaroni S, Meloni F, Solbiati L (2005) Risk of tumour seeding after percutaneous radiofrequency ablation for hepatocellular carcinoma. Br J Surg 92:856–858
64. Stigliano R, Marelli L, Yu D, Davies N, Patch D, Burroughs A (2007) Seeding risk following percutaneous approach to hepatocellular carcinoma—seeding following percutaneous diagnostic and therapeutic approaches for hepatocellular carcinoma. What is the risk and the outcome? Seeding risk for percutaneous approach of HCC. Liver Transplant 13:1603–1603
65. Imamura J, Tateishi R, Shiina S et al (2008) Neoplastic seeding after radiofrequency ablation for hepatocellular carcinoma. Am J Gastroenterol 103:3057–3062
66. Latteri F, Sandonato L, Di Marco V et al (2008) Seeding after radiofrequency ablation of hepatocellular carcinoma in patients with cirrhosis: a prospective study. Dig Liver Dis 40:684–689
67. Poon RT, Ng KK, Lam CM, Ai V, Yuen J, Fan ST (2004) Radiofrequency ablation for subcapsular hepatocellular carcinoma. Ann Surg Oncol 11:281–289

Search terms for databases.

For PubMed,

"Liver Neoplasms"[majr] AND ("Ethanol Injection"[TIAB] OR "Ablation"[TIAB] OR "Ablative therapy"[TIAB] OR "Thermal Ablation"[TIAB] OR "radiofrequency"[TIAB] OR "RFA"[TIAB] OR "PEI"[TIAB] OR "PEIT"[TIAB] OR "Ethanol/administration and dosage"[MH] OR "Injections, Intralesional"[MH] OR "microwave"[TIAB]) AND 1982/1: 2010/8[dp]) NOT Review[pt] NOT Case reports[pt]

For EMBASE

(ethanol:ab AND injection:ab) OR ablation:ab OR (ablative:ab AND therapy:ab) OR (thermal:ab AND ablation:ab) OR radiofrequency:ab OR rfa:ab OR pei:ab OR peit:ab OR microwave:ab OR (ethanol:ti AND injection:ti) OR ablation:ti OR (ablative:ti AND therapy:ti) OR (thermal:ti AND ablation:ti) OR radiofrequency:ti OR rfa:ti OR pei:ti OR peit:ti AND 'liver tumor'/exp/mj AND [english]/lim AND [1-1-1982]/sd NOT [1-8-2010]/sd NOT [review]/lim NOT [medline]/lim

Oncology

Oncology 2011;81(suppl 1):11–17
DOI: 10.1159/000333253

Published online: December 22, 2011

Hepatocarcinogenesis in Hepatitis C: HCV Shrewdly Exacerbates Oxidative Stress by Modulating both Production and Scavenging of Reactive Oxygen Species

Hidetake Fujinaga Takeya Tsutsumi Hiroshi Yotsuyanagi Kyoji Moriya
Kazuhiko Koike

Department of Internal Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Key Words
Hepatitis C · Hepatocellular carcinoma · Oxidative stress · Transgenic mouse · Core protein

Abstract
Persistent infection with hepatitis C virus (HCV) is a major risk for the development of hepatocellular carcinoma (HCC). One of the characteristics of HCV infection is the unusual augmentation of oxidative stress, which is exacerbated by iron accumulation in the liver, as observed frequently in hepatitis C patients. Using a transgenic mouse model, in which HCC develops late in life after the preneoplastic steatosis stage, the core protein of HCV was shown to induce the overproduction of reactive oxygen species (ROS) in the liver. In excessive generation of ROS, HCV affects the steady-state levels of a mitochondrial protein chaperone, i.e. prohibitin, leading to an impaired function of the mitochondrial respiratory chain with the overproduction of ROS. Insulin resistance and hepatic steatosis, which frequently accompany HCV infection, exacerbate ROS production. On the other hand, HCV compromises some of the antioxidant systems, including heme oxygenase-1 and NADH dehydrogenase quinone 1, resulting in the provocation of oxidative stress, together with ROS overproduction, in the liver with HCV infection. Thus, HCV infection not only induces ROS but also hampers the antioxidant system in the liver, thereby exacerbating oxidative stress that would facilitate hepatocarcinogenesis. Combination with the other activated pathway, including an alteration in the intracellular signaling cascade of MAP kinase, along with HCV-associated disturbances in lipid and glucose metabolism would lead to the unusual mode of hepatocarcinogenesis, i.e. very frequent and multicentric development of HCC, in persistent HCV infection.

Introduction

Approximately 200 million people are infected with hepatitis C virus (HCV) worldwide. More than two thirds of those with acute HCV infection suffer from persistent infection causing active or inactive chronic hepatitis, and approximately 30% of patients with chronic hepatitis are assumed to develop cirrhosis within their lifetime. Once HCV infection develops into cirrhosis, hepatocellular carcinoma (HCC) develops at an annual rate of 7% [1]. The strong association of oxidative stress with HCV infection has been demonstrated and can explain at least part of the clinical progression of the disease. The patho-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/11/0817–0011$38.00/0

Accessible online at:
www.karger.com/ocl

Kazuhiko Koike, MD, PhD
Department of Gastroenterology, Graduate School of Medicine
The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655 (Japan)
Tel. +81 3 5800 8800, E-Mail kkoike-tky@umin.ac.jp

genesis of chronic hepatitis C is not merely ascribed to inflammation caused by viral infection; the role of viral proteins in the pathogenesis has also been reported [2]. Of the proteins constituting HCV, the core protein in particular has various functions with respect to host cells and is closely related to oxidative stress. In this article, the relationship between HCV infection and oxidative stress is analyzed focusing on the pathological effect of the core protein of HCV, and the significance of oxidative stress in the pathogenesis of liver disease is discussed.

HCV Infection and Hepatocarcinogenesis

The mechanism underlying hepatocarcinogenesis in HCV infection is not fully understood yet. Inflammation induced by an immune response to HCV should be considered, of course, in a study on hepatocarcinogenesis in hepatitis viral infection: necrosis of hepatocytes due to chronic inflammation followed by regeneration enhances genetic aberrations in host cells, the accumulation of which culminates in HCC. This theory presupposes an indirect involvement of hepatitis viruses in HCC via hepatic inflammation. However, this context leaves us with a serious question: can inflammation alone result in the development of HCC in HCV infection with such a high incidence (90% in 15 years) or in a multicentric fashion? The other role of HCV would have to be weighed against a rare occurrence of HCC, even after the development of cirrhosis, in patients with autoimmune hepatitis in which severe inflammation in the liver persists. These backgrounds and reasonings lead to a possible activity of viral proteins for inducing neoplasia. This possibility has been evaluated by introducing genes of HCV into hepatocytes in culture with little success. One of the difficulties in using cultured cells is the carcinogenic capacity of HCV, if any, which would be weak and would take a long time to manifest itself. Actually, it takes 30–40 years for HCC to develop in individuals infected with HCV. On the basis of these viewpoints, we started to investigate carcinogenesis in chronic hepatitis C in vivo using transgenic mouse technology.

Transgenic Mouse Model for HCV-Related HCC

One of the major issues regarding the pathogenesis of HCV-associated liver lesions is whether the HCV proteins have direct effects on pathological phenotypes. For this purpose, several lines of mice have been established which are transgenic for the HCV cDNA. We have engineered transgenic mouse lines carrying the HCV genome by introducing the genes from the cDNA of the HCV genome of genotype 1b [3, 4]. Four different kinds of transgenic mouse lines are established, and they carry the core gene, envelope genes, the entire nonstructural (NS) genes, or the NS5A gene, respectively, under the same transcriptional regulatory element. Among these mouse lines, only the transgenic mice carrying the core gene developed HCC in two independent lineages [4]. The envelope gene transgenic mice did not develop HCC despite high expression levels of both E1 and E2 proteins [5], and the transgenic mice carrying the entire NS or NS5A gene developed no HCC.

Early in life, core gene transgenic mice develop hepatic steatosis, which is one of the histologic characteristics of chronic hepatitis C, along with lymphoid follicle formation and bile duct damages [6]. Thus, the core gene transgenic mouse model well reproduces the feature of chronic hepatitis C. It is important to note that no significant inflammation is observed in the liver of this animal model. Late in life, these transgenic mice develop HCC. Notably, the development of steatosis and HCC has been reproduced by other HCV transgenic mouse lines, which harbor the structural genes including the core gene [4, 7, 8]. These outcomes indicate that the core protein per se of HCV has an oncogenic potential when expressed in vivo.

Augmentation of Oxidative Stress in Hepatitis C

There is a notable feature in the localization of the core protein in hepatocytes; while the core protein predominantly exists in the cytoplasm associated with lipid droplets, it is also present in the mitochondria and nuclei [4]. On the basis of this finding, the pathways related to these two organelles, the mitochondria and nuclei, were thoroughly investigated.

One effect of the core protein is an increased production of oxidative stress in the liver. We would like to draw particular attention to the fact that the production of oxidative stress is increased in the core gene transgenic mouse model in the absence of inflammation in the liver [4]. The overproduction of oxidative stress results in the generation of deletions in the mitochondrial and nuclear DNA, an indicator of genetic damage [2].

Augmentation of oxidative stress is implicated in the pathogenesis of liver disease in HCV infection as shown by a number of clinical and basic studies [2, 9]. Reactive

oxygen species (ROS) are endogenous oxygen-containing molecules formed as normal products during aerobic metabolism. ROS can induce genetic mutations as well as chromosomal alterations and thus contribute to cancer development in multistep carcinogenesis [10, 11]. Recent studies have shown that oxidative stress is more augmented in hepatitis C than in other types of hepatitis such as hepatitis B [9].

Thus, a major role in the pathogenesis of HCV-associated liver disease has been attributed to oxidative stress augmentation, but little is known regarding the mechanism of increased oxidative stress in HCV infection. Hence, it is important to understand the mechanism of oxidative stress augmentation, in terms of both generation and scavenging of ROS, which may allow us to develop new tools of therapies for chronic hepatitis C.

Oxidative Stress and the Liver

Oxidative Stress and Reactive Oxygen

The main source of ROS in hepatocytes is the mitochondria. Outside of hepatocytes, ROS also originate from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase in Kupffer cells and inflammatory cells. A large percentage of consumed oxygen is constantly converted into ROS in the mitochondria accompanied by oxygen consumption in the electron transport system (ETS). Hepatocytes contain many mitochondria and therefore have a high ROS production. Generated ROS are very unstable and highly reactive and attack biomolecules such as DNA, lipids, and proteins. The liver not only produces much ROS but is also the center of the antioxidative effect in the form of protein synthesis. Oxidative stress refers to the oxidation-reaction-dominant state of the living body induced by an imbalance between the oxidation reaction caused by ROS and the antioxidation reaction. Main ROS include superoxide ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (HO$\cdot$). ROS are mainly produced from $\cdot O_2^-$ and converted into stable H_2O_2 through a dismutation reaction. H_2O_2 is converted into highly reactive HO$\cdot$ in the presence of a transition metal.

The Antioxidant System and Oxidative Stress Markers

Antioxidants include glutathione (GSH), thioredoxin (TRX), vitamin E, vitamin C, and β-carotene. Reactive oxygen elimination enzymes include superoxide dismutase (SOD), GSH peroxidase, heme oxygenase (HO)-1, and catalase. SOD is induced by oxidative stress and dismutates $\cdot O_2^-$ to H_2O_2 and oxygen. Catalase in peroxisomes also decomposes H_2O_2 to water and oxygen. TRX is also a protein induced by oxidative stress and is reduced via S-S binding of the substrate protein by two SH groups in TRX and acts on the H_2O_2 elimination system via peroxiredoxins. HO-1 is an inducible cytoprotective enzyme that catalyzes the initial and rate-limiting reaction in heme catabolism and cleaves prooxidant heme to form biliverdin with the release of carbon monoxide. Biliverdin is converted into bilirubin in mammals; both of these have been known to have very strong antioxidant activities.

ROS cause various forms of cellular damage. 4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA) are the peroxidation reaction products of lipids, and 8-hydroxydeoxyguanosine (8-OHdG) is the product of DNA base modification. These products serve as oxidative stress markers.

The Origin of ROS Production in HCV Infection

Then, where is the place for oxidative stress overproduction in the liver of hepatitis C patients? The core protein is mostly localized to the endoplasmic reticulum, but we and other groups have shown its localization to the mitochondria in cultured cells and transgenic mice [12]. In addition, the double structure of mitochondrial membranes is disrupted in hepatocytes of core gene transgenic mice. Evidence suggests that the core protein modulates some mitochondrial functions, including fatty acid β-oxidation, the impairment of which may induce lipid abnormalities and hepatic steatosis. In addition, the mitochondrion is an important source of ROS. In livers of transgenic mice harboring the core gene, increased ROS production has been observed [2]. A recent study found, via proteomic profiling of biopsy specimens, that impairment of key mitochondrial processes including fatty acid oxidation and oxidative phosphorylation and of the response to oxidative stress occurs in HCV-infected human liver with advanced fibrosis [13]. Therefore, it is probable that the HCV core protein affects mitochondrial functions since such pathogenesis is observed in both HCV core-transgenic mice and HCV-infected patients.

The recent progress in proteomics has opened new avenues for disease-related biomarker discovery. We performed a two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) of mitochondria isolated from HepG2 cells stably expressing the HCV core protein and

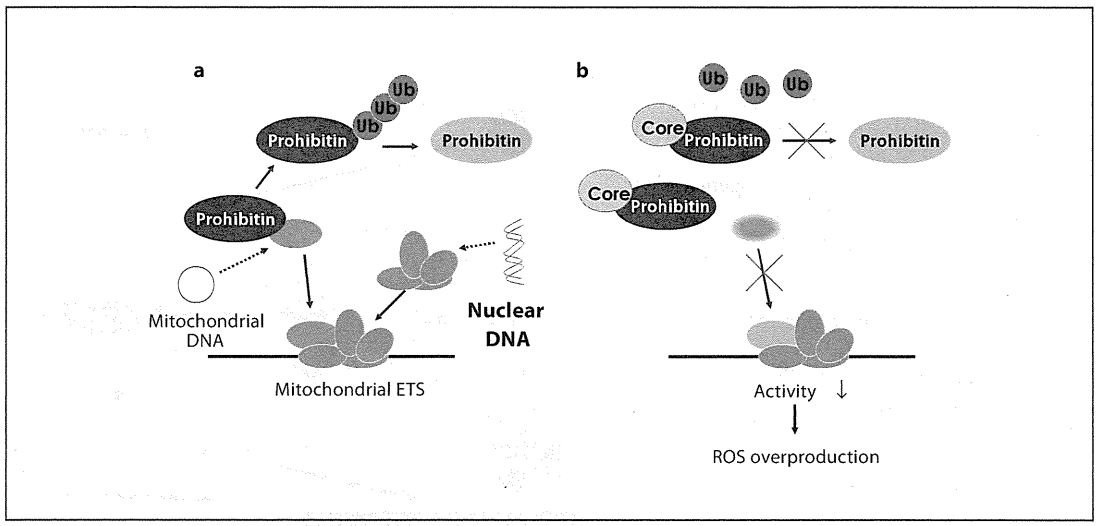

Fig. 1. The HCV core protein binds prohibitin and impairs its chaperone function leading to ROS overproduction. **a** Mitochondrial proteins consist of nuclear DNA-encoded proteins as well as mitochondrial DNA-encoded ones. Prohibitin acts as a protein chaperone for the mitochondrial proteins that are encoded by mitochondrial DNA by stabilizing newly synthesized mitochondrial translation products through direct interaction. **b** The HCV core interacts with prohibitin, disturbing its molecular chaperone function, and leads to a decrease in the activity of ETS complex IV, COX. Subunit II of COX is encoded by the mitochondrial DNA, while other subunits are encoded by the nuclear DNA. This is a new mechanism for oxidative stress overproduction in viral infection in that HCV induces mitochondrial ETS dysfunction by inhibiting chaperone function. Ub = Ubiquitin.

identified several proteins of different expressions when compared with control HepG2 cells. Among upregulated proteins in the core-expressing cells, we focused on prohibitin, which functions as a mitochondrial protein chaperone, and found that the core protein interacts with prohibitin and represses the interaction between prohibitin and subunit proteins of cytochrome c oxidase (COX), which may lead to decreases in the expression level of the proteins and in COX activity.

Prohibitin, a mitochondrial protein chaperone, was identified as an upregulated protein in core-expressing cells. Prohibitin is a ubiquitously expressed and highly conserved protein that was originally determined to play a predominant role in inhibiting cell cycle progression and cellular proliferation by attenuating DNA synthesis [14]. It is present in the nucleus and interacts with transcription factors that are important in cell cycle progression. In core-expressing cells, prohibitin was also detected in the nucleus and its expression level was also higher than that in control Hepswx cells or HepG2 cells. Mitochondrial prohibitin acts as a protein chaperone by stabilizing newly synthesized mitochondrial translation products through direct interaction [15]. We examined the interaction between prohibitin and the mitochondrially encoded subunit II of COX and found a suppressed interaction between these proteins in core-expressing cells. In addition, there are several studies that showed the association of prohibitin with the assembly of mitochondrial respiratory complex I as well as complex IV (COX) [15] (fig. 1). Complex I also consists of both nuclear- and mitochondrial-DNA-encoded subunits; therefore, it is probable that the assembly and function of complex I are impaired by the core protein. In respect to the complex I function, we previously found a decreased complex I activity in core-expressing cells. Other groups have also shown that complex I activity is decreased in cultured cells [16]. Based on these findings, the interaction between prohibitin and the core protein may impair the function of complex I as well as complex IV, leading to an increase in ROS production. In fact, the suppression of prohibitin function has been shown to result in an increased production of ROS [17], a phenomenon observed in the core-expressing cells used in this study as well as in the liver of core-gene transgenic mice [2]. Interestingly, Shelly Lu et al. [18] recently reported that the liver-specific deletion of prohibitin resulted in morphological abnormality and HCC.

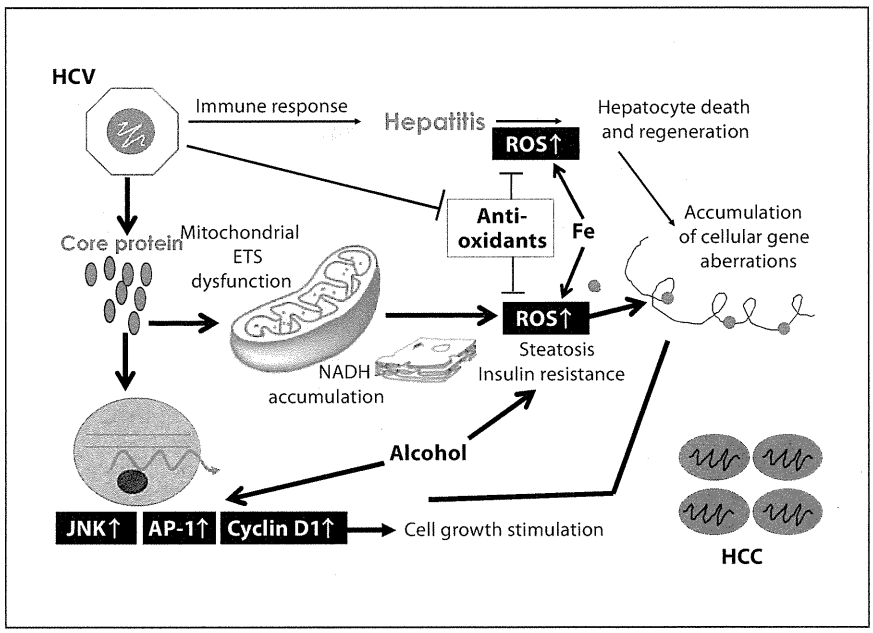

Fig. 2. Molecular pathogenesis of HCC development in HCV infection. Inflammation should contribute to hepatocarcinogenesis by producing genetic aberrations via continual cell death and regeneration. In the case of HCV infection, the virus itself contributes to hepatocarcinogenesis via two pathways. In one pathway, the core protein acts on the function of the mitochondrial ETS, leading to the overproduction of oxidative stress. The core protein also compromises some antioxidants and exacerbates ROS generation. Fe accumulation is an aggravating factor. The presence of steatosis and insulin resistance augments oxidative stress production. The other pathway is the modulation of cellular gene expression and signal transduction including the JNK pathway, which would give a growth advantage to hepatocytes. The combination of these alterations would escalate the development of HCC in HCV infection.

This is a new mechanism for ROS overproduction in viral infection in that HCV induces mitochondrial dysfunction through the inhibition of chaperone function in the mitochondria [19].

HCV Compromises the Antioxidant System

As discussed above, chronic hepatitis C is characterized by its prominent augmentation of oxidative stress. Related to this, iron accumulation in the liver has been shown to aggravate the oxidative stress as shown by the increase in the amount of DNA adducts in the liver [2, 9]. Iron is accumulated in the liver of HCV core gene transgenic mice [20]. The accumulation of iron observed in the liver of the core gene transgenic mice fed with normal chow corroborates the observation in chronic hepatitis C patients [9, 10]. Then, the impact of iron overloading on the oxidant/antioxidant system was examined using this mouse model and cultured cells. Iron overloading caused the induction of ROS as well as antioxidants. However, some of the key antioxidant enzymes, including HO-1 and NADH dehydrogenase quinone 1 (NDQ-1), were not augmented sufficiently by iron overloading, while other antioxidant enzymes such as catalase and GST were augmented more strongly in the iron-overloaded core gene transgenic mice than in the iron-overloaded control or non-iron-overloaded core gene transgenic mice. The attenuation of iron-induced augmentation of HO-1 was also confirmed in HepG2 cells expressing the core protein. HO-1 catalyzes the initial and rate-limiting reaction in heme catabolism and cleaves prooxidant heme to form biliverdin, which is converted into bilirubin in mammals; both of these have been known to have very strong antioxidant activities [21]. In addition, HO-1 has been also suggested to be a central antioxidant in conditions of GSH depletion [22]. Thus, HO-1 is an essential protective endogenous mechanism against oxidative stress, particularly in the case of iron overload. Therefore, it is probable that the attenuation of HO-1 and NQO-1 would hamper the antioxidant system and lead to a robust production of oxidative stress in HCV infection.

Thus, HCV infection not only induces ROS but also hampers antioxidant activation in the liver, thereby exacerbating oxidative stress that would facilitate hepatocarcinogenesis.

Conclusion

Pathways other than oxidative stress provocation in HCV-related hepatocarcinogenesis are alteration of the expression of cellular genes and modulation of intracellular signaling pathways. For example, tumor necrosis factor (TNF)-α and interleukin-1β have been found transcriptionally activated [23]. The mitogen-activated pro-

tein kinase (MAPK) cascade, which is involved in numerous cellular events including cell proliferation, is also activated in the liver of the core gene transgenic mouse model. In the liver prior to HCC development, only the c-Jun N-terminal kinase (JNK) route is activated. Downstream of the JNK activation, transcription factor activating protein (AP)-1 activation is markedly enhanced [23, 24]. Far downstream, both the mRNA and protein levels of cyclin D1 and cyclin-dependent kinase (CDK)4 are increased. Thus, the HCV core protein modulates the intracellular signaling pathways and gives advantage for cell proliferation to hepatocytes. The combination of these pathways that are activated in HCV infection, i.e. ROS overproduction, attenuation of antioxidants, cell growth stimulation via MAPK activation, metabolic disturbances such as hepatic steatosis, and insulin resistance [25], which are all induced by HCV itself, would contribute to hepatocarcinogenesis, together with moderate but long-lasting inflammation in chronic hepatitis C (fig. 2).

The results of our studies on transgenic mice have indicated a carcinogenic potential of the HCV core protein in vivo; thus, HCV would be directly involved in hepatocarcinogenesis. In research studies of carcinogenesis, the development of colorectal cancer is induced by the accumulation of a complete set of cellular gene mutations [26]. Their theory has been extended to the carcinogenesis of other cancers as well, called 'Vogelstein-type' carcinogenesis. On the basis of the results we obtained for the induction of HCC by the HCV core protein, we would like to introduce a different mechanism for hepatocarcinogenesis in HCV infection. We do allow multistages in the induction of all cancers; it would be mandatory for hepatocarcinogenesis that many mutations accumulate in hepatocytes. Some of these steps, however, may be skipped in the development of HCC in HCV infection to which the core protein would contribute. The overall effect achieved by expression of the viral protein would be the induction of HCC, even in the absence of a complete set of genetic aberrations, required for carcinogenesis.

By considering such a 'non-Vogelstein-type' process for the induction of HCC, a plausible explanation may be given for many unusual events which occur in HCV carriers. It no longer seem so difficult to determine why HCC develops in persistent HCV infection with an outstandingly high incidence. Our theory may also give an account of the multicentric de novo occurrence characteristics of HCC, which would be the result of persistent HCV infection.

Acknowledgements

This work was supported in part by Grant-in-Aid for Scientific Research on Priority Area from the Ministry of Education, Science, Sports and Culture of Japan; Health Sciences Research Grants of The Ministry of Health, Labour and Welfare (Research on Hepatitis).

Disclosure Statement

The authors have nothing to disclose.

References

1 Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, et al: Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2,215 patients. J Hepatol 1998;28:930–938.

2 Moriya K, Nakagawa K, Santa T, Shintani Y, Fujie H, Miyoshi H, et al: Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res 2001;61:4365–4370.

3 Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, et al: Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol 1997;78:1527–1531.

4 Moriya K, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Matsuura Y, et al: Hepatitis C virus core protein induces hepatocellular carcinoma in transgenic mice. Nat Med 1998;4:1065–1068.

5 Koike K, Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, et al: Sialadenitis resembling Sjögren's syndrome in mice transgenic for hepatitis C virus envelope genes. Proc Natl Acad Sci USA 1997;94:233–236.

6 Bach N, Thung SN, Schaffner F: The histological features of chronic hepatitis C and autoimmune chronic hepatitis: a comparative analysis. Hepatology 1992;15:572–577.

7 Machida K, Cheng KT, Lai CK, Jeng KS, Sung VM, Lai MM: Hepatitis C virus triggers mitochondrial permeability transition with production of reactive oxygen species, leading to DNA damage and STAT3 activation. J Virol 2006;80:7199–7207.

8 Naas T, Ghorbani M, Alvarez-Maya I, Lapner M, Kothary R, De Repentigny Y, et al: Characterization of liver histopathology in a transgenic mouse model expressing genotype 1a hepatitis C virus core and envelope proteins 1 and 2. J Gen Virol 2005;86:2185–2196.

9 Farinati F, Cardin R, De Maria N, Della Libera G, Marafin C, Lecis E, Burra P, Floreani A, Cecchetto A, Naccarato R: Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis. J Hepatol 1995;22:449–456.

10 Fujita N, Sugimoto R, Ma N, Tanaka H, Iwasa M, Kobayashi Y, Kawanishi S, Watanabe S, Kaito M, Takei Y: Comparison of hepatic oxidative DNA damage in patients with chronic hepatitis B and C. J Viral Hepat 2008;15:498–507.

11 Kato J, Kobune M, Nakamura T, Kuroiwa G, Takada K, Takimoto R, Sato Y, Fujikawa K, Takahashi M, Takayama T, Ikeda T, Niitsu Y: Normalization of elevated hepatic 8-hydroxy-2'-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. Cancer Res 2001;61:8697–8702.

12 Suzuki R, Sakamoto S, Tsutsumi T, Rikimaru A, Tanaka K, Shimoike T, et al: Molecular determinants for subcellular localization of hepatitis C virus core protein. J Virol 2005; 79:1271–1281.
13 Diamond DL, Jacobs JM, Paeper B, Proll SC, Gritsenko MA, Carithers RL Jr, et al: Proteomic profiling of human liver biopsies: hepatitis C virus-induced fibrosis and mitochondrial dysfunction. Hepatology 2007;46: 649–657.
14 Mishra S, Murphy LC, Nyomba BL, Murphy LJ: Prohibitin: a potential target for new therapeutics. Trends Mol Med 2005;11:192–197.
15 Nijtmans LG, de Jong L, Artal Sanz M, Coates PJ, Berden JA, Back JW, et al: Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins. EMBO J 2000;19:2444–2451.
16 Piccoli C, Scrima R, Quarato G, D'Aprile A, Ripoli M, Lecce L, et al: Hepatitis C virus protein expression causes calcium-mediated mitochondrial bioenergetic dysfunction and nitro-oxidative stress. Hepatology 2007;46: 58–65.
17 Theiss AL, Idell RD, Srinivasan S, Klapproth JM, Jones DP, Merlin D, et al: Prohibitin protects against oxidative stress in intestinal epithelial cells. FASEB J 2007;21:197–206.
18 Ko KS, Tomasi ML, Iglesias-Ara A, French BA, French SW, Ramani K, Lozano JJ, Oh P, He L, Stiles BL, Li TW, Yang H, Martínez-Chantar ML, Mato JM, Lu SC: Liver-specific deletion of prohibitin 1 results in spontaneous liver injury, fibrosis, and hepatocellular carcinoma in mice. Hepatology 2010;52: 2096–2108.
19 Tsutsumi T, Matsuda M, Aizaki H, Moriya K, Miyoshi H, Fujie H, Shintani Y, Yotsuyanagi H, Miyamura T, Suzuki T, Koike K: Proteomics analysis of mitochondrial proteins reveals overexpression of a mitochondrial protein chaperone, prohibitin, in cells expressing hepatitis C virus core protein. Hepatology 2009;50:378–386.
20 Moriya K, Miyoshi H, Shinzawa S, Tsutsumi T, Fujie H, Goto K, Shintani Y, Yotsuyanagi H, Koike K: Hepatitis C virus core protein compromises iron-induced activation of antioxidants in mice and HepG2 cells. J Med Virol 2010;82:776–792.
21 Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN: Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043–1046.
22 Oguro T, Hayashi M, Nakajo S, Numazawa S, Yoshida T: The expression of heme oxygenase-1 gene responded to oxidative stress produced by phorone, a glutathione depletor, in the rat liver; the relevance to activation of c-jun terminal kinase. J Pharmacol Exp Ther 1998;287:773–778.
23 Tsutsumi T, Suzuki T, Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, et al: Intrahepatic cytokine expression and AP-1 activation in mice transgenic for hepatitis C virus core protein. Virology 2002;304:415–424.
24 Tsutsumi T, Suzuki T, Moriya K, Shintani Y, Fujie H, Miyoshi H, et al: Hepatitis C virus core protein activates ERK and p38 MAPK in cooperation with ethanol in transgenic mice. Hepatology 2003;38:820–828.
25 Koike K, Tsutsumi T, Yotsuyanagi H, Moriya K: Lipid metabolism and pathogenesis of liver disease in hepatitis C viral infection. Oncology 2010;78(suppl 1):24–30.
26 Kinzler KW, Vogelstein B: Lessons from hereditary colorectal cancer. Cell 1996;87:159–170.

Hepatol Int (2011) 5:767–773
DOI 10.1007/s12072-011-9255-1

ORIGINAL ARTICLE

Influence of serum HBV DNA load on recurrence of hepatocellular carcinoma after treatment with percutaneous radiofrequency ablation

Tadashi Goto · Haruhiko Yoshida · Ryosuke Tateishi · Kenichiro Enooku · Eriko Goto · Takahisa Sato · Takamasa Ohki · Ryota Masuzaki · Jun Imamura · Shuichiro Shiina · Kazuhiko Koike · Masao Omata

Received: 26 December 2009 / Accepted: 10 January 2011 / Published online: 25 January 2011

Abstract
Background High serum load of hepatitis B virus (HBV) deoxyribonucleic acid (DNA) is a strong risk factor of hepatocellular carcinoma (HCC) development, independent of hepatitis B e antigen, serum alanine aminotransferase level, and liver cirrhosis. We evaluated whether serum HBV DNA load is associated with the risk of recurrence of HBV-related HCC treated with radiofrequency ablation (RFA).
Methods The study population was 69 consecutive patients with HBV-related HCC treated locally completely with RFA between January 2000 and September 2007. The risk factors for HCC recurrence were analyzed based on laboratory data, including serum HBV DNA load, together with tumor size and number using univariate and multivariate proportional hazard regression analyses.
Results HCC recurrence was observed in 42 of 69 patients during the median observation period of 1.5 years. Cumulative recurrence rates at 1, 3, and 5 years were 26.5, 57.8, and 74.3%, respectively. In univariate analysis, albumin (<3.5 g/dl), platelet count ($<150 \times 10^3/mm^3$), prothrombin activity (PT) (<70%), Child-Pugh class B, serum HBV DNA load (>4.0 log10 copies/ml), and tumor number (>3) were associated with the recurrence at $p \leq 0.15$. Multivariate Cox regression analysis with stepwise variable selection showed that the tumor number (risk ratio, 4.63; 95% CI, 1.50–14.25, $P = 0.0076$), low PT (3.39, 1.52–5.78, $P = 0.0029$), and high HBV DNA load (2.67, 1.16–6.14, $P = 0.021$) were independent risk factors for HCC recurrence.
Conclusion Serum HBV DNA load is associated with the risk of recurrence of HBV-related HCC after RFA.

Keywords Hepatitis B virus · Hepatocellular carcinoma · Recurrence · Radiofrequency ablation

Abbreviations

HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
RFA	Radiofrequency ablation
HBsAg	Hepatitis B surface antigen
HCV-Ab	Hepatitis C virus antibody
CT	Computed tomography
HBeAg	Hepatitis B e antigen
HBeAb	Hepatitis B e antibody
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
PLT	Platelet count
PT	Prothrombin activity
AFP	Alpha-fetoprotein
AFP-L3	Lens culinaris agglutinin A-reactive fraction of AFP
DCP	Des-gamma-carboxy prothrombin

T. Goto (✉) · H. Yoshida · R. Tateishi · K. Enooku · E. Goto · T. Sato · T. Ohki · R. Masuzaki · J. Imamura · S. Shiina · K. Koike · M. Omata
Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: tadagotou-gi@umin.ac.jp

Introduction

Hepatitis B virus (HBV) infection is essentially an endemic disease and the areas with high prevalence include East and Southeast Asia and sub-Saharan Africa [1]. HBV infection

is the second leading cause of hepatocellular carcinoma (HCC) in Japan, preceded by hepatitis C virus infection [2]. Currently two distinct strategies have been successfully employed against HBV infection: vaccination to prevent neonatal vertical infection and sexually transmitted horizontal infection, and oral antiviral nucleot(s)ide analogs to suppress viral replication in chronically infected patients [1, 3]. Nevertheless, sequelae of chronic HBV infection, especially HCC and liver failure, still remain a major health problem [4, 5].

HBV is a DNA virus in the Hepadna family, whose replication cycle includes the step of RNA-dependent DNA polymerization (reverse transcription). It has been indicated that a portion of HBV DNA can be integrated into host genome through reverse transcription and the integration may be causative, at least in part, of HBV-related HCC [6–8]. Serum HBV DNA load, considered to reflect the degree of viral replication in hepatocytes, has been shown to be a strong indicator of the risk of HBV-related HCC [9]. Lamivudine, a reverse transcriptase inhibitor, reportedly reduces the risk of HCC development, as well as liver decompensation, in chronic hepatitis B patients [10].

Short-term prognosis of HCC has been much improved owing to advances in diagnostic imagings and therapeutic modalities, such as surgical resection and medical ablation [11, 12]. However, long-term prognosis of HCC is not satisfactory, primarily because intrahepatic recurrence is extremely frequent. Reported risk factors for recurrence include tumor-related factors, such as the number and size of nodules, liver factors, and virus factors, including immune responses by the host [13–15]. Since HBV viral load can be attenuated with potential anti-viral agents, such as entecavir, it is clinically important to evaluate the effects of viral load on recurrence.

The frequent recurrence of HCC has been explained, in part, by metachronous multicentric carcinogenesis, or de novo HCC in the remaining liver that still harbours the virus [16, 17]. Consequently, HBV DNA load may affect the risk of intrahepatic recurrence after curative locoregional treatment of HBV-related HCC.

Radiofrequency ablation (RFA), a recently introduced minimally invasive treatment of HCC using thermal energy converted from radiofrequency current, is applicable to a wider range of patients in terms of background liver function than in surgery [12, 18]. Locoregional therapeutic efficacy of RFA has been well confirmed [12, 19, 20]. It is sometimes suspected, however, that RFA may be more prone to intrahepatic recurrence than surgical resection because intrasegmental microscopic metastasis, if any, can be removed by anatomical systemic resection but not by RFA.

It may not be easy to discern whether an individual HCC recurrence is due to de novo carcinogenesis or intrahepatic metastasis. Nevertheless, if higher serum HBV DNA load is associated with higher incidence of intrahepatic recurrence, de novo carcinogenesis is indicated to be a major mechanism of HCC recurrence also after RFA. This will encourage the use of post-RFA antiviral therapy to prevent HCC recurrence, a most urgent task in current HCC treatment. However, there were no reports on the effects of serum HBV DNA load on the recurrence of HBV-related HCC after RFA, which we sought to elucidate in the present study.

Patients and methods

Patients

From January 2000 to September 2007, we treated with RFA 832 treatment-naïve patients with HCC at the Department of Gastroenterology, the University of Tokyo Hospital, Japan. Among them, 82 patients were positive for hepatitis B surface antigen (HBsAg). Excluding 11 patients who were also positive for hepatitis C virus antibody (HCV-Ab) and 2 who could not undergo a curative RFA, a total of 69 consecutive patients with HBV related-HCC were enrolled in this study. No patients had autoimmune hepatitis or primary biliary cirrhosis as comorbidity. Six patients drank equivalent of 80 g or more ethanol a day.

RFA was selected either because patients were considered not suitable for resection because of impaired liver function, number and distribution of tumors, or cardiopulmonary comorbidities, or because they voluntarily preferred ablation with informed consent despite surgery also being feasible. Written informed consent for receiving RFA was obtained from each patient, and retrospective analysis of related clinical data were approved by the investigator and ethics committee of the University of Tokyo Hospital (approval number, 2058).

HCC diagnosis, radiofrequency ablation, and follow-up

HCC was diagnosed based on typical findings on dynamic computed tomography (CT), where hyper attenuation in the arterial phase with washout in the late phase was considered diagnostic [21]. Most nodules were also confirmed histopathologically with ultrasound-guided biopsy. The pathological grade of tumor differentiation was made based on Edmondson–Steiner criteria [22]. Stage of fibrosis and grade of activity were histologically assessed according to the criteria of Desmet et al. [23] using biopsy specimens of noncancerous liver tissue.

Indication criteria for RFA were as follows: total bilirubin concentration <3 mg/dL; platelet count (PLT) $\geq 50 \times 10^3/mm^3$; and PT $\geq 50\%$. Patients with portal vein