

HEPATOCELLULAR CARCINOMA AMONG ATOMIC BOMB SURVIVORS: SIGNIFICANT INTERACTION OF RADIATION WITH HEPATITIS C VIRUS INFECTIONS

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We conducted a nested case-control study within the cohort of Japanese survivors of the 1945 atomic bombings to study the joint effects of HBV and HCV with radiation on the risk of HCC. Among subjects who received autopsies during 1954–1988, we analyzed archival tissue samples for 238 pathologically confirmed HCC cases and 894 controls who died from diseases other than liver cancer. Using logistic regression and adjusting for potential confounders and other factors, we found a statistically significant, supermultiplicative interaction between A bomb radiation and HCV in the etiology of HCC. Compared to subjects who were negative for HCV and radiation, ORs of HCC for HCV-positive subjects showed a statistically significant, greater than multiplicative increase for liver irradiation exposures in the second (>0.018 – 0.186 Sv, $p = 0.04$) and third (>0.186 Sv, $p = 0.05$) tertiles of non-zero radiation exposure but not for first tertile exposure (>0 – 0.018 Sv, $p = 0.86$). Limiting analysis to subjects without cirrhosis, HCV-infected subjects were at 58.0-fold (95% CI 1.99– ∞) increased risk of HCC per Sv of radiation exposure ($p = 0.017$), a supermultiplicative interaction between radiation and HCV that was not found among subjects with cirrhosis ($p = 0.67$). We found no evidence of interaction between HBV infection and radiation exposure in the etiology of HCC, regardless of cirrhosis status ($p = 0.58$). We conclude that among survivors of the nuclear bombings of Hiroshima and Nagasaki, subjects who were both HCV-positive and radiation-exposed were at a significantly, supermultiplicatively increased risk of HCC without concurrent cirrhosis.

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Key words: radiation; hepatitis B; hepatitis C; hepatocellular carcinoma

Although HBV and HCV are the most important causes of HCC worldwide, each elevating risk of this cancer >10 -fold,¹ no previous study of ionizing radiation and liver cancer has taken either virus into account. We address the question of whether HBV and HCV infections might affect the relationship between acute exposure to radiation and HCC. This is important to improve our understanding of the mechanisms of hepatocarcinogenesis underlying one of the world's most common and most deadly cancers. It is also important in addressing discrepancies in the results of studies of acute radiation exposure and liver cancer, where some cohort studies conducted in areas of high HBV or HCV prevalence show significant risk elevations of liver cancer while others conducted in low-prevalence areas do not.

Studies of liver cancer risks in atomic bomb survivors have consistently shown that exposure to low-LET ionizing radiation significantly increases liver cancer mortality rates² and primary liver cancer incidence, 85% of which is HCC.^{3,4} In contrast, Western studies of radiotherapy patients have just as consistently shown liver cancer risks to not be elevated after acute radiation

exposure to substantially higher doses during medical treatment.^{5–8}

Previous studies have found synergy between hepatitis viruses and other risk factors, including (i) a supermultiplicative (or greater than multiplicative) relationship between HBV and aflatoxin exposure,^{9–12} (ii) a moderate interaction between HCV and smoking^{13,14} and (iii) a superadditive and submultiplicative relationship of both HBV and HCV with alcohol.¹⁵ A meta-analysis of 32 studies concluded that the joint effects of HBV and HCV in the etiology of HCC is between additive and multiplicative.¹

Because HBV and HCV infection rates are higher in Japan than in the Western countries, where studies have shown no relationship between acute radiation exposure and liver cancer, and because the liver appears to be especially prone to the interactive effects of multiple risk factors, our goal was to determine if radiation effects on HCC were increased by these infections.

MATERIAL AND METHODS

Selection of cases and controls

The RERF LSS cohort is a group of 120,321 atomic bomb-exposed and unexposed persons who were official residents of Hiroshima or Nagasaki in 1945 and who were alive and residing there at the time of censuses conducted in 1950–1952.^{2,16}

From a collection of archival tissue samples and clinical records for 7,647 LSS cohort members autopsied in 1954–1988 in Hiroshima and Nagasaki, we assembled material for subjects whose

Abbreviations: CI, confidence interval; DS86, dosimetry system 1986; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LET, linear energy transfer; LSS, Life Span Study; OR, odds ratio; RERF, Radiation Effects Research Foundation; RR, relative risk; Sv, sievert.

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cause of death was either liver cancer or one of several diseases frequently confused with liver cancer, including cirrhosis, chronic hepatitis, pancreatic cancer and gallbladder or biliary disease. This material was reviewed by a panel of 3 pathologists, as described in more detail elsewhere.^{17,18} In brief, each pathologist independently reviewed medical material for each subject, and the panel then discussed each subject and reached consensus opinions on type of liver cancer, if present, cirrhosis status and HBV status (based on results of Orcein and immunohistochemical staining). The panel accepted 238 autopsied cases as primary HCC. Potential, nonautopsied cases of liver cancer were also identified through tumor registries; and tissue samples, when available, were stained and reviewed. For controls, we chose 894 persons without liver cancer from the group of 7,647 who were determined not to have liver cancer. Prior to case review, controls were matched to all potential cases on sex and city of residence at the time of the bombings and further selected to achieve balance with the potential cases on radiation exposure, age at death and, to the extent possible given the restricted period of the autopsy program, year of death. As described in more detail elsewhere,¹⁹ a weighted distance score between all potential cases and controls was computed, and controls were selected who were closest to the cases on this distance score, individually within sex and city strata. Originally, slightly more than twice as many controls as potential cases were selected, to end up with about a 2:1 ratio after allowing for the possibility of unusable tissue. However, because there was no suitable source of comparable controls with nonautopsy liver tissue, we excluded all nonautopsied cases from the present analysis. We also excluded autopsied liver cancer cases who were determined by the pathology review panel to have cholangiocarcinoma and other subtypes of liver cancer other than HCC. In summary, our study was limited to 238 autopsied, pathologist-confirmed HCC cases and 894 autopsied, pathologist-reviewed controls, where controls were sampled according to sex, city, radiation dose, age at death and year of death but not necessarily matched to cases.

Histologic classification was made in accordance with the standards proposed by the WHO.²⁰ Histologic evidence of cirrhotic changes was obtained from nonneoplastic liver tissue, and the changes were characterized according to the 4 types proposed by Anthony *et al.*²¹

Determination of HBV and HCV status

To detect HBsAg, the pathology panel reviewed tissue slides stained with Orcein²² and slides stained by anti-HBV immunohistochemical material (LSAB kit, Universal K681; Dako, Carpinteria, CA). Slides were prepared from nonneoplastic, formalin-fixed and paraffin-embedded liver tissues. To increase the accuracy of HBV testing, we also used PCR to test archival tissues from nontumor areas of the liver for the presence of genes encoding HBV antigens, as described elsewhere.²³ DNA was extracted from 5- μ m-thick sections of tissue, and 3 separate HBV loci were amplified, the S, pre-C and X regions, using the following specific primer pairs for each locus: S region, MD03, 5'-CTTGATCCTATGGGAGTGG-3' and MD06, 5'-CTCAAGCTTCATCATCATATA-3'; pre-C region, P20, 5'-AGGCATAAATTGGTCTGCGC-3' and M1, 5'-ACGAGAGTAACTCCACAGTAGCTCC-3'; and X region, XP22, 5'-CCAGCAATGTCAACGACCG-3' and XM0, 5'-ATTATGCCTACAGCCTCC-3'. If any of these 3 regions of the HBV genome could be amplified or if either the Orcein or immunohistochemical stain was positive for HBV, the subject was considered positive for HBsAg. According to a WHO consensus opinion, the presence of HBsAg indicates active HBV infection.²⁴

Agreement between the Orcein and immunohistochemical staining methods for HBV was good (κ statistic = 0.87, n = 1,014). Assuming that persons positive on at least one of the staining tests were HBV-positive, agreement between PCR tests for HBV and staining tests was also good (κ = 0.95, n = 1,017). Using radioimmunoassay procedures and commercially available reagents, we performed HBsAg tests on 51 subjects for whom frozen (-80°C)

or freeze-dried serum samples were available. All 45 persons whose serum was negative for HBsAg were negative by tissue-based tests; 5 (83%) of the 6 persons whose serum was HBsAg-positive were positive by tissue-based staining or PCR. Because tissue staining and PCR were more likely to falsely classify HBV-positive subjects as negative instead of falsely classifying negative subjects as positive, we classified anyone testing HBV-positive by staining or PCR as positive to maximize the sensitivity of our tests. We classified everyone else testing negative as negative since the specificity of our tests was high.

To determine HCV status, we extracted RNA from a single 5- μ m-thick section of paraffin-embedded liver tissue.²⁵ The methods of detecting the HCV genome and ensuring the integrity of mRNA in each sample and primer set have been described in detail elsewhere.²⁶ Briefly, the 5'-untranslated region of the HCV genome was amplified using our specific primer sets. After RT-PCR amplification of HCV, positive samples were identified by hybridizing with a radiolabeled oligomer probe that recognizes a sequence between the 2 primers. RNA integrity was assessed by amplification of *c-BCR* mRNA between 2 sequential exons with an intervening intron by RT-PCR.

We obtained and tested frozen serum samples (-80°C) from 43 subjects for whom HCV results of tissue-based RT-PCR tests were available. These samples were tested, under code, for HCV antibodies by ELISA-2 using commercially available reagents and by qualitative RT-PCR. Fifteen (65%) of the 23 subjects testing HCV-negative by both serum tests were negative by RT-PCR; the others tested positive by tissue-based RT-PCR. Of the 20 subjects testing positive by either serum test, 14 (70%) also tested positive by tissue-based RT-PCR, the other 6 being negative. Because serum samples were available for just a small fraction of subjects for whom tissue samples were available, analysis of the case-control study was based entirely on RT-PCR of archival samples of liver tissue.

Radiation exposure

Measures of liver irradiation from the atomic bombs were derived from the DS86.²⁷ This dosimetry system provides estimates of liver dose based on physical calculations of neutron particle and γ -ray bomb yields, interviews with cohort members about their locations and shielding by buildings and terrain during the bombings and estimates of radiation shielding by body tissue. We allowed for the differential effectiveness of γ -rays and neutron particles using a relative biologic effectiveness weighing factor of 10, multiplying the neutron dose by this number and adding it to the γ dose, as described in more detail elsewhere.⁴ Among controls, the mean γ and neutron liver doses were 0.104 Gy (SD = 0.275) and 0.0007 Gy (SD = 0.003), respectively; among cases, these values were 0.123 Gy (SD = 0.335) and 0.0011 Gy (SD = 0.004).

Statistical methods

We used unconditional logistic regression to calculate ORs and 95% CIs of HCC for the risk factors under investigation, adjusting simultaneously for HBV and HCV and for the potential confounders on which controls were selected. Adjustment was made by adding main effects terms to the logistic regression model. Because viral hepatitis has a different relationship with HCC depending on whether cirrhosis is present or not,²⁸ we included cirrhosis status in statistical models or calculated ORs separately for subjects with and without cirrhosis. Because controls were selected to have a distribution of radiation exposure similar to that of cases, we either calculated ORs of HCC for exposure groups after separating subjects based on 4 exposure strata (see below) or estimated a trend with radiation dose using the mean radiation doses from the 4 strata in logistic models. Under DS86, kerma doses up to 0.005 Gy (<0.003 Sv liver dose) were recorded as 0; thus, some subjects in our 0-dose radiation group may have received inconsequential liver doses of 0.001–0.002 Sv.

To examine the joint effects of A bomb radiation and viral hepatitis, we constructed 4 exposure groups: unexposed (0 dose) and 3 groups of exposed (non-0 dose) subjects based on tertiles of radiation exposure calculated for exposed controls. Tertiles among the exposed (non-0 dose) were $>0-0.018$ Sv, $>0.018-0.186$ Sv and >0.186 Sv (Table I). We created radiation-virus interaction terms, which were the product of the mean dose in each exposure stratum and a 0 or 1 variable, representing exposure to HBV or HCV. We used unconditional logistic regression to calculate ORs and 95% CIs for these interaction terms, taking into account HBV and HCV, cirrhosis status and the control sampling factors of radiation dose, year of death, age at death, city and sex were also calculated *p* values for each interaction term included in logistic models to determine whether or not we could reject the null hypothesis that interaction was multiplicative in favor of the alternative hypothesis of supermultiplicative interaction, setting 0.05 as the level of statistical significance.

To calculate ORs for combined HCV and tertile-specific radiation exposures, we added the HCV main effect parameter to each HCV-mean dose interaction parameter and calculated the exponent of the result. The 95% CIs for these ORs were calculated using the formula for the SE of a sum of 2 variables: square root [variance ($b_{\text{HCV}} + b_{\text{interaction}}$) = variance (b_{HCV}) + variance ($b_{\text{interaction}}$) + $2 \times$ covariance ($b_{\text{HCV}}, b_{\text{interaction}}$)]. The CI was the exponent of [the sum of the 2 parameters \pm 1.96 times SE]. The expected values under the multiplicative model for these ORs could not be directly estimated because of control selection on radiation dose. To estimate the main effect for radiation, we used the latest excess RR estimates of liver cancer for A bomb radiation,³ adjusted to the mean doses in the tertiles. These OR estimates were multiplied by the OR for HCV.

We calculated profile likelihood 95% CIs and likelihood ratio *p* values. Model fit was evaluated using the Hosmer and Lemeshow

goodness-of-fit test. SAS version 6.12 was used to perform all analyses (SAS Institute, Cary, NC).

RESULTS

Table I shows the distribution of sex, city, liver irradiation, age at death, year of death and HBV/HCV status among cases and controls. Among cases, 75.5% had cirrhosis compared to 7.1% of controls.

HCV and radiation

We found the integrity of RNA, as measured by the percentage of *c-BCR* mRNA amplifiable by RT-PCR of liver samples, to be 59.5%. We determined HCV status for 61.7% of controls and 62.6% of cases.

Table II presents a comparison of 2 logistic models examining the joint effects of HCV and liver irradiation in the etiology of HCC. The full model included cirrhosis status, the control selection factors, HBV/HCV status and the HCV-radiation interaction term; the reduced model excluded the nonsignificant factors radiation dose, age at death and sex. As shown in Table II, both the full and reduced models showed borderline statistically significant results for the HCV-radiation interaction term. Under the reduced model, the OR of HCC among the HCV-infected increased 5.7-fold per Sv increase in radiation exposure (95% CI 0.86-37.91). The corresponding increase under the full model was 10.0 per Sv increase, but the 95% CI for this OR was much wider with the inclusion of the additional factors.

As shown in Table III, when analysis was limited to the 528 subjects without cirrhosis, there was a statistically significant, positive interaction between liver irradiation and HCV (*p* = 0.017). Thus, we can reject the null hypothesis that the joint effect is multiplicative and accept the alternative hypothesis that it is

TABLE I—CONTROL SELECTION AND POTENTIAL RISK FACTORS FOR AUTOPSIED SUBJECTS DYING FROM HCC (CASES) OR FROM DISEASES OTHER THAN LIVER CANCER (CONTROLS), HIROSHIMA AND NAGASAKI, JAPAN, 1954–1988

Characteristic	Controls		Cases	
	Number	%	Number	%
Sex				
Female	261	29.2	69	29.0
Male	633	70.8	169	71.0
Age at death (years)				
20–39	31	3.5	4	1.7
40–59	235	26.3	79	33.2
60–90	628	70.2	155	65.1
Decade of death				
1950s	26	2.9	3	1.3
1960s	350	39.2	60	25.2
1970s	453	50.7	82	34.5
1980s	65	7.3	93	39.1
City of residence at time of bombing				
Hiroshima	576	64.4	163	68.5
Nagasaki	318	35.6	75	31.5
Liver irradiation level (mean Sv) ¹				
Unknown	52	5.8	13	5.5
0 (0)	465	52.0	127	53.4
Tertile 1 (0.009)	124	13.9	27	11.3
Tertile 2 (0.071)	127	14.2	35	14.7
Tertile 3 (0.686)	126	14.1	36	15.1
HBV				
Negative	730	81.7	148	62.2
Positive	42	4.7	62	26.0
Unknown	122	13.6	28	11.8
HCV				
Negative	510	57.0	82	34.4
Positive	42	4.7	67	28.3
Unknown	342	38.3	89	37.4
Total	894		238	

¹Under the current dosimetry system (DS86), liver doses up to 0.003 Sv are recorded as 0. Radiation exposures within tertiles were 1, $>0-0.018$ Sv; 2, $>0.018-0.186$ Sv; and 3, >0.186 Sv.

TABLE II – JOINT EFFECTS OF ATOMIC BOMB RADIATION AND HCV INFECTIONS ON RISKS OF HCC, AUTOPSIED CASES AND CONTROLS, 1954–1988

Risk factor	Full model ¹ (<i>n</i> = 670)		Reduced model ² (<i>n</i> = 693)	
	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
HCV infection (yes/no)	5.9 (2.68–13.39)	0.0001	6.2 (2.80–13.93)	0.0001
HBV infection (yes/no)	5.8 (2.68–12.67)	0.0001	5.5 (2.60–12.00)	0.0001
HCV–radiation interaction term (per Sv with HCV infection) ³	10.0 (0.87–137.76)	0.072	5.7 (0.86–37.91)	0.065
Cirrhosis	45.2 (23.66–91.86)	0.0001	44.8 (23.78–89.35)	0.0001

¹Also adjusted for the study's control selection factors: radiation exposure (*p* = 0.50), year of death (*p* = 0.0001), age at death (*p* = 0.78), city (*p* = 0.0001) and sex (*p* = 0.40).²Additionally adjusted only for statistically significant factors: year of death and city.³Mean liver irradiation level for exposure category (as shown in Table I) multiplied by a binary (0, 1) term representing HCV infection status.

TABLE III – JOINT EFFECTS OF ATOMIC BOMB RADIATION AND HCV INFECTIONS ON RISKS OF HCC ACCORDING TO PRESENCE OF CIRRHOSIS, AUTOPSIED CASES AND CONTROLS, 1954–1988

Risk factor	Cirrhosis not present (<i>n</i> = 528) ¹		Cirrhosis present (<i>n</i> = 142) ¹	
	OR (95% CI) ²	<i>p</i> ²	OR (95% CI) ²	<i>p</i> ²
HCV infection (yes/no)	3.5 (0.98–10.96)	0.054	9.0 (2.86–35.77)	<0.0001
HBV infection (yes/no)	14.2 (4.93–40.90)	<0.0001	3.0 (1.07–9.66)	0.036
HCV–radiation interaction term (per Sv with HCV infection) ³	58.0 (1.99–∞)	0.017	0.4 (0.0007–59.26)	0.67

¹Numbers of subjects for whom information available for all factors included in logistic models: no cirrhosis, 30 cases and 498 controls; cirrhosis-positive, 108 cases and 34 controls.²Also adjusted for the study's control selection factors: radiation exposure, year of death, age at death, city and sex.³Mean liver irradiation level for exposure category (as shown in Table I) multiplied by a binary (0, 1) term representing HCV infection status.

TABLE IV – JOINT EFFECTS OF ATOMIC BOMB RADIATION AND HCV INFECTIONS ON RISKS OF HCC, AUTOPSIED CASES AND CONTROLS, 1954–1988¹

HCV/radiation status	Controls		Cases		Parameter estimate (95% CI)	<i>p</i>	OR ² (95% CI)
	Number	%	Number	%			
HCV ⁻ and no radiation	239	85.6	41	39.8			1.0
HCV ^{+/-} and 1 Sv liver irradiation ³	—	—	—	—			1.8 (1.32–2.43)
HCV ⁺ and no liver irradiation	25	9.0	34	33.0	1.55 (0.689–2.438)	0.0005	4.7 (1.99–11.44)
HCV ⁺ and tertile 1 liver irradiation ⁴	4	1.4	6	5.8	1.55 + 0.19 (-1.847–2.344)	0.86	5.7 (0.76–43.0)
HCV ⁺ and tertile 2 liver irradiation ⁴	2	0.7	10	9.7	1.55 + 2.46 (0.127–4.915)	0.04	55.1 (5.9–523.1)
HCV ⁺ and tertile 3 liver irradiation ⁴	9	3.2	12	11.6	1.55 + 1.81 (0.058–3.677)	0.05	28.7 (5.8–141.2)
Total	279	100%	103	100%			

¹The logistic model included the following factors HCV and HBV infection status, the 3 interaction terms, cirrhosis status and the study's control selection factors: radiation exposure, year of death, age at death, city and sex.²Corresponding ORs, 95% CIs and parameter *p* values among subjects negative for cirrhosis were 2.9 (0.66–10.8; *p* = 0.12), (no radiation, HCV⁺), undefined (*p* = 0.99, tertile 1 radiation, HCV⁺), 41.3 (3.9–436.8; *p* = 0.05, tertile 2 radiation, HCV⁺) and 61.2 (6.5–580.1; *p* = 0.02, tertile 3 radiation, HCV⁺).³Because radiation dose was a factor for control selection, the RR of HCC for radiation exposure could not be estimated. The RR of liver cancer of 1.8 per Sv is taken from the latest cohort analysis of liver cancer among A bomb survivors,³ an analysis that did not adjust for HCV (about 9% of the cohort was HCV-infected).³³⁴Means and ranges of liver irradiation exposures for tertiles are listed in Table I.

greater than multiplicative. HCV-infected, noncirrhotic persons were at 58-fold increased risk of HCC per Sv of liver irradiation, though the 95% CI for this OR was wide (1.99–∞). There was no significant interaction between HCV and radiation in the etiology of HCC accompanied by cirrhosis (OR = 0.4, *p* = 0.67). These logistic models controlled for all 5 control selection factors, including liver irradiation, as well as for HCV and HBV. Although HCV infections were significantly associated with both noncirrhotic and cirrhotic HCC, the virus was a stronger risk factor for HCC among subjects with cirrhosis (ORs = 3.5 and 9.0, respectively).

As shown in Table IV, compared to subjects who were negative for both HCV and liver irradiation, HCV-positive subjects with no liver irradiation were at 4.7-fold greater risk of HCC (*p* = 0.0005) after adjusting for cirrhosis. ORs and 95% CIs of HCC for HCV-positive subjects with tertiles 1, 2 and 3 radiation exposure were 5.7 (0.76–43.0), 55.1 (5.9–523.1) and 28.7 (5.8–141.2), respectively. Based on the excess RR of 0.8 per Sv liver irradiation found in the most recent cohort analysis of liver cancer risk among A bomb survivors³ and under the multiplicative model, we would expect an RR of 7.3 [4.7 × (1 + 0.8 × 0.686)] for the joint effects

of HCV and the mean dose of liver irradiation in the highest exposure group (tertile 3). When we restricted analysis to subjects without cirrhosis and controlled for the factors listed in Table IV, the corresponding ORs and 95% CIs were 2.9 (0.66–10.8; no radiation, HCV⁺), undefined (*p* = 0.99; tertile 1 irradiation, HCV⁺), 41.3 (3.9–436.8; tertile 2 irradiation, HCV⁺) and 61.2 (6.5–580.1; tertile 3 irradiation, HCV⁺). Among subjects without cirrhosis under the multiplicative model, we would expect an RR of 3.6 [2.9 × (1 + 0.8 × 0.686)] for the joint effects of HCV and the mean liver irradiation level in tertile 3.

HBV and radiation

HBV status was determined for 86.4% of controls and 88.2% of cases. We found no evidence of interaction between HBV and liver irradiation in the etiology of HCC. The *p* value for the HBV–radiation term was 0.58, adjusting for HCV, HBV, cirrhosis status and the 5 control selection factors, including liver irradiation. This *p* value was 0.30 when cirrhosis status was excluded from the model. In contrast to HCV, HBV infection was a stronger risk factor for HCC among those without cirrhosis (ORs 14.2 and 3.0, respectively) (Table III).

DISCUSSION

We found that HCV infection combined with liver irradiation significantly elevated HCC risks after controlling for the effects of HCV and liver irradiation alone, as well as for cirrhosis status and other factors. HCC risks were 28.7-fold higher ($p = 0.05$) among HCV-infected persons exposed to the highest one-third of non-0 liver radiation doses compared to those negative for liver irradiation and HCV. In contrast, under a conservative multiplicative model, which uses the possibly inflated RR at 1 Sv of 1.8 reported by a cohort study of liver cancer in HCV-positive and -negative A bomb survivors,³ the expected RR for this comparison would be 7.3. HCC risks were also significantly elevated in HCV-infected subjects exposed to the middle-third of non-0 liver radiation doses ($p = 0.04$); HCC risks were not significantly elevated for HCV-infected subjects with lower radiation exposures ($p = 0.86$). Among subjects without cirrhosis, HCV-infected subjects with tertile 2 and 3 radiation exposures were at 41.3-fold ($p = 0.05$) and 61.2-fold ($p = 0.02$) greater risk of HCC, respectively, again in comparison to HCV-negative and radiation-unexposed subjects and after subtracting out the effects of liver irradiation and HBV and HCV infection alone. Under a multiplicative model, the expected OR for tertile 3 radiation exposure and HCV infection would be 3.6. Thus, our results are consistent in indicating a greater than multiplicative relationship between HCV and liver irradiation in the etiology of HCC, which is especially pronounced among subjects without cirrhosis.

In contrast, we found no increased risks of HCC for liver irradiation among HBV-positive persons after factoring out the effects of HBV and liver irradiation alone on hepatocarcinogenesis.

The major difficulty in conducting this research was the limited number of cases and controls for whom radiation exposures were known and liver tissue samples were available to allow pathology review and assessment of cirrhosis and viral hepatitis. To increase statistical power, we selected 2 controls per potential case. After pathology review and restriction of cases to subjects with pathology-confirmed HCC who, like controls, had received autopsies, the study included nearly 4 times as many controls as cases. To further increase statistical power, we selected controls to have a distribution of radiation exposures similar to that of cases, to increase the number of controls exposed to higher levels of liver irradiation, a number likely to be too low had they been selected randomly. Matching the distributions of radiation dose for cases and controls has been demonstrated to be effective at increasing power to detect statistical interaction.²⁹ Nevertheless, our data are fairly sparse, as can be seen by the wide CIs accompanying many of the ORs. Our finding of no interaction in the etiology of HCC accompanied by cirrhosis should be interpreted cautiously since this analysis was based on just 142 subjects with both cirrhosis and complete exposure information. In comparison, our risk estimates for subjects without cirrhosis were based on exposure assessments of 528 subjects and have greater statistical power. Although we consistently found a statistically significant, greater than multiplicative interaction between liver irradiation and HCV in the etiology of HCC, the degree to which risks of this cancer are increased by these joint exposures must be viewed as poorly quantified by this study due to sparse data.

A second problem of our study was the unequal distribution of cases and controls by year of death because the autopsy program was more active in earlier years of cohort follow-up and the incidence of HCC increased in the cohort over time. To address this problem, we insured that the range of years of death was identical for both cases and controls (1954–1988), and we included year of death in all analyses. According to Yoshizawa,³⁰ HCV was widespread in Japan in the 1950s due to use of illegal drugs after World War II. We found that 9.2% of subjects dying in the 1960s were HCV-infected, which also suggests that HCV was present in Japan this early. Since 868 controls and 235 cases died after 1959, a large number of subjects would have been alive when

HCV was present in this population. Our findings suggest that risks of HCV-induced HCC increase with radiation dose. Since we controlled for both radiation dose and year of death in all analyses and there was not a significant association between year of death and radiation dose, it appears unlikely that the difference in years of death of cases and controls could account for our results.

Tissue-based measures of HBV showed better agreement with serum-based measures in our limited validation than did tissue-based measures of HCV. Radiation exposure measures were based on interviews conducted and measurements made in the early 1950s at the beginning of cohort follow-up. Cirrhosis and HCC disease classifications were based on review by 3 pathologists of the ample liver tissue samples obtained during autopsies, and the pathologists were required to reach consensus opinions. Our results might be affected if the somewhat older tissue samples for controls were more likely to falsely test negative for HCV than the tissues of cases. However, RNA integrity was assessed by amplification of *c-BDR* mRNA; and when this RNA, which is present in all living cells, could not be amplified, samples were discarded. Success rates for HCV testing were similar for cases and controls (62.6% and 61.7%, respectively). Thus, the misclassification of HCV, as well as of the other risk factors and of cirrhosis status, that did occur is likely to have occurred randomly among cases and controls, thereby biasing OR estimates toward a finding of no association.

The time of radiation exposure of the A bomb survivor cohort is known precisely. Although HCV infections can become chronic regardless of the age at which they occur and therefore could have occurred at any time, there are several reasons to suspect that many infections followed A bomb irradiation in 1945: (i) mean age at bombing of HCV-infected cases was 33.8 years, and mean age at death was 66.1 years (thus, on average, about half their lifetimes were lived after the bombings); (ii) trauma from the explosions was associated with blood transfusions that were not screened for HCV; (iii) percentages of HCV-positive subjects increased with decade of death (from 9.2% in the 1960s to 14.6% in the 1970s to 30.4% in the 1980s). Therefore, it appears reasonable, albeit speculative, to suggest that in many instances hepatocytes were mutated by A bomb radiation but the process of carcinogenesis did not continue until subjects were infected with HCV and the virus started its cycle of hepatocyte destruction and regeneration. If cell mutation or epigenetic change occurred before the cellular proliferation associated with HCV infection rather than after it, the process of carcinogenesis might progress to HCC without going through the stage of cirrhosis. Thus, our failure to find interaction between radiation and HCV in the etiology of HCC accompanied by cirrhosis may not mean that no such interaction occurs. The explanation may lie in a time sequence of radiation exposure followed by HCV infection that is specific to the A bomb survivor cohort.

Our findings that the effect of acute radiation exposure on HCC risk was significantly increased by HCV infection may explain the consistently negative findings from large-scale mortality and incidence studies of acute radiation effects on liver cancer conducted in areas of low HCV prevalence. The findings from those studies are in conflict with the consistently elevated liver cancer risks found among the Hiroshima and Nagasaki survivors who were also acutely exposed to ionizing radiation but generally at much lower mean levels.^{2–4} No excess liver cancer risk was found in U.S., U.K. or European populations exposed to high levels of acute liver irradiation during treatment of ankylosing spondylitis (mean liver dose 2.1 Gy),⁶ peptic ulcer (mean liver dose 4.6 Gy),⁵ benign gynecologic bleeding disorders (mean liver dose 0.21 Gy)³¹ and cervical cancer (mean liver dose 1.5 Gy).⁷ In comparison, mean liver doses in our study were 0.14 Sv for cases and 0.11 Sv for controls. (Although doses in the radiotherapy studies were in Gy, these would be equal to adjusted equivalent doses in Sv because X radiation has identical properties to γ radiation of the same energy, and both have relative biologic effectiveness weighing factor of 1.)

According to the review of Wasley and Alter,³² HCV prevalence in the United States is about 1.8%, with lower prevalences reported for Western Europe (0.2–0.5%) and the lowest HCV prevalences found in the United Kingdom and Scandinavia (0.01–0.1%). In contrast, the prevalence of HCV in the A bomb survivor cohort is 4–5 times higher than in the United States and 80 or more times higher than in the United Kingdom, ranging from 7.8% for the controls in our study to 8.9% in an earlier clinical study of 6,121 A bomb survivors.³³

Our findings suggest that excess RRs of primary liver cancer for radiation among HCV-negative A bomb survivors are lower than the previous mortality study excess risk estimates of 0.27 per Gy² and the incidence study excess risk estimates of 0.66 per Sv⁴ and 0.81 per Sv³ because these studies did not take into account HCV status and, conversely, radiation risks would be higher than this among HCV-positive persons. We did not find significant elevations in HCC risk for HCV-infected subjects in the lowest tertile of non-0 A bomb liver irradiation exposure (≤ 0.018 Sv), but risks were significantly elevated for HCV-positive subjects with mid-tertile and top-tertile radiation exposures. Supermultiplicative interaction was found both when we included cirrhotic and noncirrhotic subjects together in the analysis and adjusted for cirrhosis and when we limited analysis to noncirrhotic subjects.

Roles of viral hepatitis and other risk factors in hepatocarcinogenesis

Generally in epidemiologic studies of cancer the strongest interactions between risk factors are found when 2 agents play active roles at different steps in the carcinogenic process. In HCC, synergistic or supermultiplicative interactions between risk factors have been reported when 1 agent is primarily associated with genetic alteration of hepatocytes and the other with cellular proliferation and liver regeneration leading to clonal expansion.^{12,34,35} Ionizing radiation has long been known for its ability to cause mutations and malignant transformation of cells.³⁶

HBV has been called a "complete carcinogen" and appears to be involved in multiple steps in oncogenic progression to HCC.³⁷ Persistent HBV infection causes inflammation, increased cell turnover and cirrhosis. In addition, the HBV genome may be incorporated into the chromosomes of hepatocytes and may then cause genomic instability as a result of point mutations, deletions, translocations and rearrangements at multiple sites.³⁸ In contrast, the role of HCV in HCC appears to primarily relate to its ability to cause inflammation, cellular injury and cirrhosis,³⁸ leading to cellular proliferation and hepatocyte regeneration, a function that higher cirrhosis rates for HCV indicates is more associated with HCV than with HBV.³⁹ Unlike both HBV infection and radiation, HCV, an RNA virus, does not directly damage or integrate into cellular DNA; and if it has a direct oncogenic effect in causing HCC, it would have to exert it from an extrachromosomal position.⁴⁰

In terms of HBV, HCV and HCC, Donato *et al.*¹ concluded in their meta-analysis that these viruses act through both common and different pathways in the process of hepatocarcinogenesis. It is reasonable to hypothesize that these viruses interact in dually infected persons in a superadditive and sub-, not super-, multiplicative fashion¹ because HBV acts as a cell mutagen and HBV and HCV have overlapping roles in causing both liver cell regeneration

and cellular proliferation, leading to clonal expansion. In contrast, the supermultiplicative interaction between acute radiation and HCV that we report suggests that the roles played by these agents overlap to a lesser degree, with acute radiation acting as an agent of mutagenesis or epigenetic change and HCV primarily acting as an agent of cellular proliferation. Lack of evidence of an interaction between HBV and acute radiation in the etiology of HCC suggests that these factors do not play active roles at different steps in hepatocarcinogenesis, perhaps with radiation's role as a mutagen being overshadowed by HBV's strong mutagenic qualities.

Several studies showing a strong link between chronic radiation exposure and liver cancer were conducted in areas of low HCV prevalence, including thorotrast studies in Germany,⁴¹ Denmark⁴² and Portugal.⁴³ Although these studies were limited to liver cancer in general, comparisons with tumor registry data show a statistically significant association between HCC and chronic α -radiation exposure resulting from thorotrast administration.⁴⁴ However, chronic and acute exposure to radiation appear to have a different association with liver cirrhosis, a frequent cause of liver cell proliferation. While Andersson *et al.*⁴⁵ reported an 11-fold increase in cirrhosis among Danish patients exposed to thorotrast, we found no increased risk of cirrhosis for A bomb irradiation.⁴⁶ Thus, while chronic exposure to high radiation doses may cause both genetic alteration and cellular proliferation of hepatocytes leading to HCC, acute radiation exposure at the levels experienced by the A bomb survivors may only cause the first of these events; thus, acute, unlike chronic, radiation exposure may require an additional risk factor for progression to HCC.

In summary, our study suggests that ionizing radiation and HCV infection interact to supermultiplicatively increase the risk of HCC. Based on our results, we conclude that ionizing radiation at the exposure levels studied significantly increases HCC risks in the presence of HCV when cirrhosis is not concurrently detected. Our results fit into a pattern with other studies of interaction in hepatocarcinogenesis, with synergistic or greater than multiplicative interactions in HCC being reported when subjects are exposed both to agents such as radiation that primarily cause genetic alteration and to agents such as HCV and heavy drinking that cause hepatocyte destruction, triggering liver cell regeneration. Our results suggest that persons infected with HCV may be particularly sensitive to radiation exposure and *vice versa*. Future studies of liver irradiation and HCC should take into account HCV infection status.

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