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A Prospective Follow-up Study of the Association of Radiation Exposure and the Incidence of Haemorrhagic Stroke among Atomic Bomb Survivors in Hiroshima and Nagasaki

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Article summary

1) Article focus

- Use of medical radiotherapy has increased in recent decades.
- Whether the consequence includes an increased risk of cardiovascular disease is unknown.
- Our purpose is to examine the association between radiation exposure and the incidence of stroke among atomic bomb survivors in Japan.

2) Key messages

- Risk of haemorrhagic stroke increased with rising radiation exposure for both sexes, although effects in women were less apparent until doses exceeded a threshold at 1.3 Gy.
- Radiation exposure was unrelated to ischaemic stroke.

3) Strengths and Limitations

- This report provides information on the incidence of stroke using data from clinical examinations and mortality records following a structured research protocol.
- Measurement of radiation exposure adheres to a precise system of

quantification.

- While best attempts were made to properly classify strokes outcomes, diagnostic uncertainties persist.

Competing interesting None.

Abstract

Objective

Use of medical radiotherapy has increased markedly in recent decades. Whether the consequence includes an increased risk of cardiovascular disease remains to be determined. Our purpose is to examine the association between radiation exposure and the incidence of stroke among Japanese atomic-bomb survivors.

Methods

Radiation exposure from the atomic bombing was assessed in 9,515 subjects (34.8% male) with 24-year follow-up from 1980. Stroke events and the underlying cause of death were reviewed to confirm the first-ever stroke, and subtypes (ischaemic and haemorrhagic events) were categorized based on established criteria according to the definitions of typical/atypical stroke symptoms. All subjects were free of prevalent stroke at the baseline of 1980. Radiation dose exposure was estimated for each individual.

Results

The mean radiation dose was 0.4±0.6 gray (Gy) (range: 0-3.5 Gy). During the study period, 235 haemorrhagic and 607 ischaemic events were identified. For men, after adjusting for age and concomitant risk factors, the risk of haemorrhagic stroke rose consistently from 11.6 to 29.1/10,000 person-years as doses increased from <0.05 to ≥2 Gy (p=0.009). Incidence also rose within the dose range <1 Gy (p=0.004) with no dose threshold. In women, the risk of haemorrhagic stroke rose with increasing radiation exposure but not until doses reached a threshold of 1.3 Gy (95% confidence interval 0.5-2.3). Among women, for doses <1.3 Gy, differences in stroke risk were modest (13.5/10,000 person-years) while it increased to 20.3/10,000 person-years for doses that ranged from 1.3 to <2.2 Gy and to 48.6/10,000 person-

years for doses that were higher (p=0.002). In both sexes, dose was unrelated to ischaemic stroke.

Conclusion

While the risk of haemorrhagic stroke increases with rising radiation exposure for both sexes, effects in women are less apparent until doses exceed a threshold at 1.3 Gy.

Introduction

Worldwide use of radiographic procedures in medicine has increased markedly in recent decades.[1-3] While health benefits are thought to outweigh the risk of adverse side-effects, increased use of radiotherapy, particularly in the age range <65 years,[2] raises concerns over the promotion of a variety of adverse health outcomes, most notably cancer. Although equivocal, data from patient samples and occupational studies suggest that a corresponding rise could also occur in the incidence of circulatory disease and asymptomatic atherosclerosis.[4-11] Based on mail surveys and vital statistics records from the Japanese atomic-bomb survivors Life Span Study (LSS), evidence indicates that radiation >0.5 Gy increases the risk of all-stroke death (1950-2003).[12] Associations that include gender effects and stroke subtypes, however, have not been clearly identified. Our purpose is to examine the association between radiation and stroke incidence among atomic-bomb survivors in the Adult Health Study (AHS) from the Radiation Effects Research Foundation (RERF) over two decades (1980-2003). Stroke outcomes include morbidity and mortality from haemorrhagic and ischaemic events after adjustment for several concomitant risk factors.

Methods

Study Population

In 1950, the Atomic Bomb Casualty Commission (now the RERF) established the Life Span Study (LSS) of 120,321 survivors of the atomic bombings of Hiroshima and Nagasaki, Japan.[13] Follow-up is limited to periodic mail surveys and mortality outcomes from vital statistics data. In 1958, a series of comprehensive physical examinations was launched with enrollment of 19,961 of the LSS participants into the AHS. In the AHS, examinations have been given biennially with informed consent and approval from the RERF Ethics Committee. The AHS biennial health examinations provide clinical information complementary to death and tumor registries data. The AHS includes individuals exposed to a broad range of doses to enhance detection of radiation effects on a variety of disease outcomes. Participation rate has ranged from 70 to 90% throughout the examination cycles. For the current report, follow-up began at examinations that were given in 1980. From that time, subjects were followed for incident stroke over a 24-year period (until the end of 2003). Of the eligible 11,231 participants, 208 prevalent stroke (35 were haemorrhagic, 117 were ischaemic, and 56 were of unknown origin), and 1,508 without dose information were excluded. The final sample includes 9,515 AHS participants.

Radiation Dosimetry

Estimation of radiation dose exposure for each individual was based on an updated dosimetry system that takes into account biases arising from errors in calculated doses, physical locations, and organ shielding at the time of bombing.[14] For all analyses, weighted colon doses were used in units of gray (Gy), where the dose for an individual corresponds to the total exposure in γ rays + 10× the smaller neutron dose. [14]

Stroke Ascertainment

Possible stroke events and the underlying cause of death were coded according to International Classification of Disease. Virtually all deaths are assumed accounted for based on access to a comprehensive nationwide registration of deaths in Japan. The number of missed cases of nonfatal strokes in subjects who remained alive at the close of follow-up (2003) is unknown, although with high participation across repeated AHS examinations, it is thought to be small. There is no indication of bias in the indexing of stroke by radiation exposure. All data (health exams, death certificates, and autopsy reports when available) were reviewed to confirm the first-ever stroke. Stroke was defined as an acute-onset focal neurological deficit of vascular etiology, persisting for at least 24 hours. Stroke subtypes (ischaemic and haemorrhagic events) were categorized based on established criteria that included clinical features, neuroimaging and noninvasive vascular studies, and other laboratory criteria according to the definitions of typical/atypical stroke symptoms in the World Health Organization (WHO) Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Projects.[15] Ischaemic stroke was diagnosed if there was a focal neurological deficit in the absence of haemorrhage based on neuroimaging, when the neuroimage showed an ischaemic infarct that correlated with the clinical deficit, or an ischaemic infarct was documented at autopsy. Not all diagnoses of stroke were based on neuroimaging studies. Among the cases of haemorrhagic stroke, 50.2% were diagnosed based on death certificates alone (ischaemic stroke, 49.3%; all-stroke, 49.2%). Further details regarding the stroke surveillance have been published elsewhere.[16]

Baseline Examination and Questionnaires

Baseline concomitant data included the age when follow-up began, systolic blood pressure (SBP), total cholesterol (T-CHO), body mass index (BMI), diabetes, smoking, and alcohol intake. Data on smoking and alcohol intake were collected from mail surveys that were

administered from 1978 to 1980. In the absence of such data, information was taken from mail surveys in 1965. Smoking status was defined as never, past, and current smoker. Alcohol intake was defined according to typical Japanese consumption strata in units of ethanol as nondrinker, light drinker (<34 g/d), and heavy drinker (≥34 g/d).[17] The remaining data were collected at clinical examinations that were given in 1980. In the event that an examination cycle failed to coincide with 1980, information (within 5 years) from the most recently available examination was used. Measurement of nonfasting T-CHO is described elsewhere.[18] Sitting SBP was measured in the left arm. BMI was defined as weight (kg) divided by height squared (m). A diagnosis of diabetes was based on a physician diagnosis or the use of medications for diabetes.

Statistical methods

Crude and age-adjusted incidence of haemorrhagic and ischaemic stroke in person-years of follow-up were estimated across common ranges of radiation dose based on standard analysis of covariance methods.[19] Percents and average levels of the confounding risk factors were also derived and age-adjusted based on similar techniques. To test for an independent effect of radiation on the risk of each stroke subtype, proportional hazards regression models were used with radiation dose modeled as a continuous predictor variable. Adjustments were made for age when follow-up began, SBP, and the other risk factors. Nonlinear relationships between radiation dose and the stroke risk were also considered, including a threshold analysis. For the latter, dose was modeled as $(D-\delta) \times I_{\delta}(D)$ where D is a radiation dose, δ is a threshold, and $I_{\delta}(D) = 1$ when $D \geq \delta$ and 0 otherwise. The dose that minimizes $-2 \times$ the log likelihood provides a point estimate for δ. A 95% confidence interval for δ consists of upper and lower threshold values for which $-2 \times$ the log likelihood differs from the minimum value by 3.84 (the 95th percentile from a χ^2 distribution with 1 degree of freedom). If the lower threshold value is >0 Gy, then a dose threshold is assumed to exist with 95% confidence. Although primary tests of significance were

based on radiation dose being modeled as a continuous risk factor, patterns of association are also described through the use of indicator variables that allow for the estimation of the relative hazard of stroke (and 95 percent confidence intervals) between radiation dose strata ≥0.05 Gy versus doses considered to be small (<0.05 Gy). All reported p-values were based on two-sided tests of significance. Statistical modeling and testing were based on the use of SAS software (version 9.2, SAS Institute Inc., Cary, NC, USA).

Results

Radiation exposure and study characteristics

Table 1 provides the distribution of radiation exposures, average baseline ages, and age-adjusted means and percent characteristics for the sample of men and women who were available for follow-up. The average radiation dose was similar between the sexes (0.4 ± 0.6 Gy). For men, 15.3% were exposed to radiation doses ≥1 Gy, while 46.5% were exposed to doses <0.05 Gy. For women, corresponding percents were 11.6 and 44.6%.

After adjusting for differences in baseline age across the ranges of radiation exposure, BMI declined with increased radiation exposure in men (p=0.016) but not in women. For women, higher radiation doses were more likely associated with elevated SBP (p=0.013). A similar pattern was absent in men. While not significant, T-CHO levels were highest in women who had the greatest dose exposures. For the remaining data, associations with radiation were absent.

Age-adjusted incidence of stroke by radiation exposure

During the course of follow-up, there were 235 haemorrhagic and 607 ischaemic strokes (14.0 and 36.1/10,000 person-years, respectively). The average age at the time of a stroke was 73.2 years (range: 43 - 98 years) for those that were haemorrhagic and 77.0 years (range: 48 - 100 years) for those that were ischaemic. The average follow-up time before stroke occurrence was 11.1 years (range: 3 months - 23 years) for haemorrhagic events and 11.5 years (range: 4 days - 23 years) for ischaemic events. There were an additional 84 strokes that were of unknown subtype.

Table 2 provides further details on stroke incidence that was identified according to radiation exposure at the time of bombing. For men, after adjusting for age, the incidence of haemorrhagic stroke rose consistently from 12.2 to 25.2/10,000 person years as radiation

exposure increased from <0.05 to ≥2 Gy (p=0.006). Risk of haemorrhagic stroke continued to rise with increasing doses <1 Gy (p=0.006). For women, differences in the risk of haemorrhagic events were modest for doses <2 Gy but more than tripled when doses went higher (p=0.002). There were no significant relationships between radiation and the risk of ischaemic events for either sex (table 2). Relationships to total stroke were largely determined through associations with haemorrhagic events. There were no relationships between radiation and the incidence of strokes that were of unknown origin.

Risk factor adjusted associations between haemorrhagic stroke and radiation exposure

The association between radiation and haemorrhagic events was further examined after adjustment for age, SBP, BMI, diabetes, T-CHO, cigarette smoking, alcohol drinking, and city (table 3). For men, the association was diminished but remained significant (p=0.009). For those exposed to the highest amounts of radiation (≥2 Gy), there was a 2.5-fold excess risk of stroke as compared to doses that were <0.05 Gy (29.1 versus 11.6/10,000 person-years). Risk of haemorrhagic stroke also rose with increasing doses <1 Gy (p=0.004) suggesting that the dose-response relationship in men is not entirely attributed to the excess of haemorrhagic events that were observed in the highest ranges of radiation.

For women, while a dose-response relationship across the entire range of radiation and the risk of haemorrhagic events was absent, evidence in table 2 suggests that there may exist a threshold effect. Further analyses identified a significant dose threshold at 1.3 Gy (95% confidence interval, 0.5 - 2.3 Gy) where a change occurs in the association between radiation and the risk of haemorrhagic stroke. Below the threshold, risk was unrelated to radiation, while above the threshold, it increased with rising dose. This can better be seen in table 3 for dose strata <0.05, 0.05 to <1.3, 1.3 to <2.2, and ≥2.2 Gy. For doses <1.3 Gy, differences in haemorrhagic risk were modest (14.2 and 13.0/10,000 person-years) while it increased to

20.3/10,000 person-years for doses that ranged from 1.3 to <2.2 Gy and to 48.6/10,000 person-years for doses that were higher. For men, a dose threshold was absent. In both sexes, findings do not appear to be influenced by events that occur at an early age (<55 years).

Discussion

Findings suggest that exposure to increasing radiation doses among atomic-bomb survivors beyond a threshold of 1.3 Gy is associated with an increase in the future risk of haemorrhagic stroke in women. While 1.3 Gy is only a point estimate, the lower 95% confidence limit (0.5-2.3 Gy), further suggests that there is more than 95% confidence that the true threshold is 0.5 Gy or higher. For men, the incidence of haemorrhagic stroke rose consistently with increasing exposure levels without evidence for a threshold. Even within the dose range <1 Gy, the dose-response observed in men persisted. It seems noteworthy that the timing of the radiation exposure in both sexes predated the haemorrhagic events by 35 years. Such a long latency period may be especially meaningful as the use of radiotherapy increases in younger ages, allowing for a longer period of time for a stroke to develop.[2]

Patterns of association persisted for the period that predated the 1980 baseline, during a time when enrollment in the AHS continued to be ongoing. For the 1980 baseline used in the current report, more than 97% of the AHS participants had been enrolled. The 1980 baseline also provides a uniform beginning with a fixed lag time since the bombing of Hiroshima and Nagasaki. More complete data from clinical examinations and a recently conducted mail survey were also available. Although many stroke diagnoses were based on death certificates alone (about 50%), confounding due to changes in the diagnosis of stroke through the advent of neuroimaging in the late 1970s was also thought to be minimized. With regard to the diagnostic uncertainty that is common in any large-scale study, best attempts were made for the proper classification of fatal and nonfatal strokes with the opportunity for adjudication among the study investigators. In the absence of neuroimaging, however, diagnostic limitations are difficult to avoid. In spite of evidence from Japanese samples that suggest that errors in stroke classification could be small,[20 21] subtle distinctions between primary intracerebral haemorrhage and

ischaemic events can still exist when neuroimaging is available.

There is also evidence of an excess risk of circulatory disease at low and moderate doses (<5Gy) in Japanese atomic-bomb survivors in the Life Span Study cohort [12] where follow-up began in 1950. Although there were no clinical examinations in the LSS, there was a 9% excess risk of death due to all strokes combined per unit Gy (p=0.02), and a slightly, but not significantly, higher risk for haemorrhagic events versus cerebral infarction.[12] Corroborating evidence also appears elsewhere, [5 10] while reports of uncertainty in the association between radiation and stroke are common,[11 22] most likely due to the extreme difficulties in quantifying radiation exposure in studies that often rely on limited record keeping and historical recall.

Given that the association between radiation and the risk of stroke is plausible, an explanation for the association is far from clear. In a paired comparison of 42 patients receiving radiotherapy for head and neck tumors, a significant excess in carotid intima media thickness was observed in irradiated versus non-irradiated carotid arteries,[4] suggesting the possibility for a link with ischaemic stroke through atherosclerotic damage. More direct mechanistic derangements that might explain an association with haemorrhagic stroke include fibrinoid necrosis of the small arteries and arteriole, a common underlying cause for intracerebral haemorrhage due to hypertension.[23 24] Fibrinoid necrosis in vessels is also preceded by proinflammatory cytokines and observed in late cerebral radionecrosis at radiotherapy doses less than 0.05 Gy.[25] Elevated blood pressure,[26] hypertension,[27] and inflammation (C-reactive protein and interleukin-6) among the atomic-bomb survivors [28 29] might further promote a fibrinoid necrosis link with haemorrhagic stroke. Hypertension has a greater impact on haemorrhagic stroke incidence than cerebral infarction.[16] In the absence of a clear explanation for the reported findings, further study of subclinical arteriosclerosis or biological evidences among the AHS may provide additional insight into the role that radiation has on promoting

stroke and its subtypes.

In the current report, several notable limitations need to be addressed. Whether findings apply to other ethnicities is unknown. Genetic susceptibilities may be different. The number of haemorrhagic events is also limited, and interactions with confounding data are difficult to assess. In spite of the limitations, findings from the AHS warrant consideration and further study. Attractive features include the high rate of participation between examination cycles and free access to standardized medical care and evaluation. Measurement of radiation exposure also adheres to a rigorous system of quantification.[14] Among large cohort studies, the dosimetry in the AHS is unusually precise. In conclusion, the risk of haemorrhagic stroke increases with rising radiation exposure for both sexes. In men, it seems to occur across the full range of radiation exposures, while in women, the risk becomes apparent when doses exceed a threshold at about 1.3 Gy. Given the observed latency between radiation exposure and haemorrhagic stroke in the current study, a consequence of the expanded use of radiotherapy in younger individuals[2] could be an increased opportunity for the development of adverse outcomes in later life.

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Contributors IT contributed in study design, interpreted the data, and draft writing. RDA conducted the statistical analyses, interpreted the data, and draft review. TO and TT provided the advice on the study design and contributed in the draft review. KO, MA, and SF contributed in draft review and supervised the study design. KK and MM provided supervision of the study. All authors critically revised the manuscript for important intellectual content.

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References

1. Mettler FA, Jr., Bhargavan M, Faulkner K, Gilley DB, Gray JE, Ibbott GS, et al. Radiologic and nuclear medicine studies in the United States and worldwide: frequency, radiation dose, and comparison with other radiation sources--1950-2007. *Radiology* 2009;253:520-31.
2. Bhargavan M. Trends in the utilization of medical procedures that use ionizing radiation. *Health Phys* 2008;95:612-27.
3. Mettler FA, Jr., Thomadsen BR, Bhargavan M, Gilley DB, Gray JE, Lipoti JA, et al. Medical radiation exposure in the U.S. in 2006: preliminary results. *Health Phys* 2008;95:502-7.
4. Dorresteijn LD, Kappelle AC, Scholz NM, Munneke M, Scholma JT, Balm AJ, et al. Increased carotid wall thickening after radiotherapy on the neck. *Eur J Cancer* 2005;41:1026-30.
5. Carr ZA, Land CE, Kleinerman RA, Weinstock RW, Stovall M, Griem ML, et al. Coronary heart disease after radiotherapy for peptic ulcer disease. *Int J Radiat Oncol Biol Phys* 2005;61:842-50.
6. Howe GR, Zablotska LB, Fix JJ, Egel J, Buchanan J. Analysis of the mortality experience amongst U.S. nuclear power industry workers after chronic low-dose exposure to ionizing radiation. *Radiat Res* 2004;162:517-26.
7. Ivanov VK. Late cancer and noncancer risks among Chernobyl emergency workers of Russia. *Health Phys* 2007;93:470-9.
8. Scott AS, Parr LA, Johnstone PA. Risk of cerebrovascular events after neck and supraclavicular radiotherapy: a systematic review. *Radiother Oncol* 2009;90:163-5.
9. Hauptmann M, Mohan AK, Doody MM, Linet MS, Mabuchi K. Mortality from diseases of the circulatory system in radiologic technologists in the United States. *Am J Epidemiol* 2003;157:239-48.
10. McGeoghegan D, Binks K, Gillies M, Jones S, Whaley S. The non-cancer mortality experience of male workers at British Nuclear Fuels plc, 1946-2005. *Int J Epidemiol* 2008;37:506-18.
11. Muirhead CR, O'Hagan JA, Haylock RG, Phillipson MA, Willcock T, Berridge GL, et al. Mortality and cancer incidence following occupational radiation exposure: third analysis of the National Registry for Radiation Workers. *Br J Cancer* 2009;100:206-12.
12. Shimizu Y, Kodama K, Nishi N, Kasagi F, Suyama A, Soda M, et al. Radiation exposure and circulatory disease risk: Hiroshima and Nagasaki atomic bomb survivor data, 1950-2003. *BMJ* 2010;340:b5349.
13. Preston DL, Pierce DA, Shimizu Y, Cullings HM, Fujita S, Funamoto S, et al. Effect of recent changes in atomic bomb survivor dosimetry on cancer mortality risk estimates. *Radiat Res* 2004;162:377-89.
14. Cullings HM, Fujita S, Funamoto S, Grant EJ, Kerr GD, Preston DL. Dose estimation for atomic bomb survivor studies: its evolution and present status. *Radiat Res* 2006;166:219-54.
15. Asplund K, Bonita R, Kuulasmaa K, Rajakangas AM, Schaedlich H, Suzuki K, et al. Multinational comparisons of stroke epidemiology. Evaluation of case ascertainment in the WHO MONICA Stroke Study. World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease. *Stroke* 1995;26:355-60.
16. Takahashi I, Geyer SM, Nishi N, Ohshita T, Takahashi T, Akahoshi M, et al. Lifetime risk of stroke and impact of hypertension: estimates from the adult health study in Hiroshima and Nagasaki. *Hypertens Res* 2011;34:649-54.

17. Kiyohara Y, Kato I, Iwamoto H, Nakayama K, Fujishima M. The impact of alcohol and hypertension on stroke incidence in a general Japanese population. The Hisayama Study. *Stroke* 1995;26:368-72.
18. Yamada M, Wong FL, Kodama K, Sasaki H, Shimaoka K, Yamakido M. Longitudinal trends in total serum cholesterol levels in a Japanese cohort, 1958-1986. *J Clin Epidemiol* 1997;50:425-34.
19. Lane PW, Nelder JA. Analysis of covariance and standardization as instances of prediction. *Biometrics* 1982;38:613-21.
20. Tanaka H, Ueda Y, Hayashi M, Date C, Baba T, Yamashita H, et al. Risk factors for cerebral hemorrhage and cerebral infarction in a Japanese rural community. *Stroke* 1982;13:62-73.
21. Tanaka H, Ueda Y, Date C, Baba T, Yamashita H, Hayashi M, et al. Incidence of stroke in Shibata, Japan: 1976-1978. *Stroke* 1981;12:460-6.
22. Vrijheid M, Cardis E, Ashmore P, Auvinen A, Bae JM, Engels H, et al. Mortality from diseases other than cancer following low doses of ionizing radiation: results from the 15-Country Study of nuclear industry workers. *Int J Epidemiol* 2007;36:1126-35.
23. Daigle JL, Hong JH, Chiang CS, McBride WH. The role of tumor necrosis factor signaling pathways in the response of murine brain to irradiation. *Cancer Res* 2001;61:8859-65.
24. da Costa L, Wallace MC, Ter Brugge KG, O'Kelly C, Willinsky RA, Tymianski M. The natural history and predictive features of hemorrhage from brain arteriovenous malformations. *Stroke* 2009;40:100-5.
25. Xie CH, Zhang MS, Zhou YF, Han G, Cao Z, Zhou FX, et al. Chinese medicine Angelica sinensis suppresses radiation-induced expression of TNF-alpha and TGF-beta1 in mice. *Oncol Rep* 2006;15:1429-36.
26. Sasaki H, Wong FL, Yamada M, Kodama K. The effects of aging and radiation exposure on blood pressure levels of atomic bomb survivors. *J Clin Epidemiol* 2002;55:974-81.
27. Yamada M, Wong FL, Fujiwara S, Akahoshi M, Suzuki G. Noncancer disease incidence in atomic bomb survivors, 1958-1998. *Radiat Res* 2004;161:622-32.
28. Hayashi T, Kusunoki Y, Hakoda M, Morishita Y, Kubo Y, Maki M, et al. Radiation dose-dependent increases in inflammatory response markers in A-bomb survivors. *Int J Radiat Biol* 2003;79:129-36.
29. Neriishi K, Nakashima E, Delongchamp RR. Persistent subclinical inflammation among A-bomb survivors. *Int J Radiat Biol* 2001;77:475-82.

Table 1. Distribution of radiation exposures, average baseline age, and age-adjusted means and percent characteristics for the sample of men and women available for stroke follow-up

Characteristic	Radiation exposure (Gy)			
	<0.05	0.05 to <1	1 to <2	≥2
Men (sample size)	1539	1266	376	130
Percent of sample	46.5	38.2	11.4	3.9
Baseline age* (y)	57 ±13	57 ±13	54 ±13	51 ±11
SBP (mmHg)	135 ±24	135 ±23	133 ± 20	136 ± 19
T-CHO (mmol/L)	4.53 ± 0.88	4.60 ± 0.85	4.65 ± 0.96	4.55 ± 0.96
BMI† (kg/m^2)	21.9 ± 3.0	21.8± 3.0	21.7 ± 2.9	21.5 ± 3.0
Diabetes (%)	12.6	14.6	12.5	15.5
In Nagasaki at exposure	40.8	31.1	41.2	30.2
Smoking status (%)				
Past	21.6	21.0	22.7	16.8
Current	66.6	65.6	64.2	68.5
Alcohol intake (%)				
Light (<34 g/day)	44.3	42.0	42.9	44.1
Heavy (≥34 g/day)	38.3	38.3	41.2	38.4
Women (sample size)	2765	2720	531	188
Percent of sample	44.6	43.8	8.6	3.0
Baseline age* (y)	59 ±13	60 ±13	58 ±12	56 ±12
SBP‡ (mmHg)	134 ±26	135 ±26	136 ±25	137 ±26
T-CHO§ (mmol/L)	4.94 ± 0.91	5.02 ± 0.96	5.09 ± 1.01	5.15 ± 0.93
BMI (kg/m^2)	22.8 ±3.6	22.9 ±3.5	23.0 ± 3.5	22.3 ± 3.2
Diabetes (%)	6.7	8.4	8.0	7.2
In Nagasaki at exposure‖	31.0	26.5	35.7	25.8
Smoking status (%)				
Past	5.1	4.3	5.3	7.2
Current	10.6	14.7	13.8	13.1
Alcohol intake (%)				
Light (<34 g/day)	20.7	21.3	21.3	16.2
Heavy (≥34 g/day)	0.8	1.9	1.3	0.0

Continuous variables are reported as means ± standard deviations. The remaining variables are reported as percents.

*Significant decline with radiation exposure (p<0.001)

† Significant decline with radiation exposure (p=0.016)

‡ Significant increase with radiation exposure (p=0.013)

§ Significant increase with radiation exposure (p<0.001)

||Significant increase with radiation exposure (p=0.002)

Abbreviations: SBP, systolic blood pressure; BMI, body mass index; T-CHO, total cholesterol

Table 2. Age-adjusted stroke incidence by radiation exposure.

Radiation exposure (Gy)	Sample size	Haemorrhagic		Ischaemic		Total stroke	
		Events	Incidence*	Events	Incidence*	Events	Incidence*
			Men				
<0.05	1539	33	12.2	112	40.5	154	56.1
0.05 to <1	1266	37	17.6	81	38.5	132	62.7
1 to <2	376	13	21.4	20	34.9	36	61.9
≥2	130	5	25.2	8	46.3	13	72.4
p-value†			0.006‡		0.788		0.202
Overall	3311	88	15.7	221	39.4	335	59.7
			Women				
<0.05	2765	66	13.1	173	34.4	262	52.0
0.05 to <1	2720	63	12.4	174	33.9	264	51.6
1 to <2	531	8	9.3	33	39.8	46	54.9
≥2	188	10	41.9§	6	27.9	19	85.7‖
p-value			0.098		0.930		0.155
Overall	6204	147	13.1	386	34.4	591	52.7

*Incidence rate per 10,000 person-years

†The p-value is a test for trend with dose modeled as a continuous variable.

‡For men, risk of haemorrhagic stroke continued to rise with increasing doses <1 Gy (p=0.006).

§Risk of haemorrhagic stroke in women is higher for doses ≥2 Gy versus lower doses (p=0.002).

‖Risk of total stroke in women is higher for doses ≥2 Gy versus lower doses (p=0.027).

Table 3. Risk factor adjusted incidence and relative hazards of haemorrhagic stroke by radiation exposure

Radiation exposure (Gy)	Incidence	Relative hazard (95% CI)
	Men	
<0.05	11.6	reference
0.05 to <1	17.7	1.5 (0.8, 2.7)
1 to <2	20.2	1.7 (0.7, 4.1)
≥2	29.1	2.5 (0.8, 7.3)
p-value†	0.009‡	
	Women	
<0.05	14.2	reference
0.05 to <1.3	13.0	0.9 (0.6, 1.4)
1.3 to <2.2	20.3	1.4 (0.6, 3.7)
≥2.2	48.6	3.5 (1.4, 9.0)
p-value	0.002	

Incidence rate per 10,000 person-years and relative hazards are adjusted for age, systolic blood pressure, body mass index, diabetes, total cholesterol, cigarette smoking, alcohol drinking, and city.

†The p-value is a test for trend with dose modeled as a continuous variable. For women, a dose threshold model is used with a dose threshold at 1.3 Gy (95% CI, 0.5– 2.3 Gy).

‡For men, risk of haemorrhagic stroke continued to rise with increasing doses <1 Gy (p=0.004).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3, 4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	1, 4, 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*8-9*
Bias	9	Describe any efforts to address potential sources of bias	7, 14
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9, 10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9-10
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	7-8
		(*d*) If applicable, explain how loss to follow-up was addressed	7-8
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11 and Table1
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) Summarise follow-up time (eg, average and total amount)	10
Outcome data	15*	Report numbers of outcome events or summary measures over time	10 and Table 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11-12, and Table 2-3
		(*b*) Report category boundaries when continuous variables were categorized	9 and Tables 1-3
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Table2
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	14
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

A Prospective Follow-up Study of the Association of Radiation Exposure with Fatal and Non-Fatal Stroke among Atomic Bomb Survivors in Hiroshima and Nagasaki (1980-2003)

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A Prospective Follow-up Study of the Association of Radiation Exposure **with Fatal and Non-Fatal Stroke** among Atomic Bomb Survivors in Hiroshima and Nagasaki **(1980-2003)**

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Running title: Stroke incidence in atomic-bomb survivors

Key words: Stroke, radiation, atomic-bomb survivors

Article summary

1) Article focus

- Use of medical radiotherapy has increased in recent decades.
- Whether the consequence includes an increased risk of cardiovascular disease is unknown.
- Our purpose is to examine the association between radiation exposure and the incidence of stroke among atomic bomb survivors in Japan.

2) Key messages

- Risk of haemorrhagic stroke increased with rising radiation exposure for both sexes, although effects in women were less apparent until doses exceeded a threshold at 1.3 Gy.
- Radiation exposure was unrelated to ischaemic stroke.

3) Strengths and Limitations

- This report provides information on the incidence of stroke using data from clinical examinations and mortality records following a structured research protocol.
- Measurement of radiation exposure adheres to a precise system of

quantification.

- While best attempts were made to properly classify strokes outcomes, diagnostic uncertainties persist.

Competing interesting None.

Abstract

Objective

Use of medical radiotherapy has increased markedly in recent decades. Whether the consequence includes an increased risk of cardiovascular disease remains to be determined. Our purpose is to examine the association between radiation exposure and the incidence of stroke among Japanese atomic-bomb survivors.

Methods

Radiation exposure from the atomic bombing was assessed in 9,515 subjects (34.8% male) with 24-year follow-up from 1980. Stroke events and the underlying cause of death were reviewed to confirm the first-ever stroke**. S**ubtypes (ischaemic and haemorrhagic events) were categorized based on established criteria according to the definitions of typical/atypical stroke symptoms. All subjects were free of prevalent stroke at the baseline of 1980. Radiation dose exposure was estimated for each individual.

Results

Overall mean radiation dose **(± standard deviation) in units of gray (Gy)** was 0**.38 ±** 0**.58** (range: 0-3.5). During the study period, 235 haemorrhagic and 607 ischaemic events were identified. For men, after adjusting for age and concomitant risk factors, the risk of haemorrhagic stroke rose consistently from 11.6 to 29.1/10,000 person-years as doses increased from <0.05 to ≥2 Gy (p=0.009). Incidence also rose within the dose range <1 Gy (p=0.004) with no dose threshold. In women, the risk of haemorrhagic stroke rose with increasing radiation exposure but not until doses reached a threshold of 1.3 Gy (95% confidence interval 0.5-2.3). Among women, for doses <1.3 Gy, differences in stroke risk were modest (13.5/10,000 person-years) while it increased to 20.3/10,000 person-years for doses that ranged from 1.3 to <2.2 Gy and to

48.6/10,000 person-years for doses that were higher (p=0.002). In both sexes, dose was unrelated to ischaemic stroke.

Conclusion

While the risk of haemorrhagic stroke increases with rising radiation exposure for both sexes, effects in women are less apparent until doses exceed a threshold at 1.3 Gy.

Introduction

Worldwide use of radiographic procedures in medicine has increased markedly in recent decades.[1-3] While health benefits are thought to outweigh the risk of adverse side-effects, increased use of radiotherapy, particularly in the age range <65 years,[2] raises concerns over the promotion of a variety of adverse health outcomes, most notably cancer. Although equivocal, data from patient samples and occupational studies suggest that a corresponding rise could also occur in the incidence of circulatory disease and asymptomatic atherosclerosis.[4-11] Based on mail surveys and vital statistics records from the Japanese atomic-bomb survivors Life Span Study (LSS), evidence indicates that radiation >0.5 Gy increases the risk of all-stroke death (1950-2003).[12] Associations that include gender effects and stroke subtypes, however, have not been clearly identified. Our purpose is to examine the association between radiation and stroke incidence among atomic-bomb survivors in the Adult Health Study (AHS) from the Radiation Effects Research Foundation (RERF) over two decades (1980-2003). Stroke outcomes include morbidity and mortality from haemorrhagic and ischaemic events after adjustment for several concomitant risk factors.

Methods

Study Population

In 1950, the Atomic Bomb Casualty Commission (now the RERF) established the Life Span Study (LSS) of 120,321 survivors of the atomic bombings of Hiroshima and Nagasaki, Japan.[13] Follow-up is limited to periodic mail surveys and mortality outcomes from vital statistics data. In 1958, a series of comprehensive physical examinations was launched with **the establishment of the AHS cohort consisting** of 19,961 **subjects from among** the LSS **subjects**. In the AHS, examinations have been given biennially with informed consent and approval from the RERF Ethics Committee. The AHS biennial health examinations provide clinical information complementary to death and tumor registries data. The AHS includes individuals exposed to a broad range of doses to enhance detection of radiation effects on a variety of disease outcomes. Participation rate has ranged from 70 to 90% throughout the examination cycles. For the current report, follow-up began at examinations that were given in 1980. From that time, subjects were followed for incident stroke over a 24-year period (until the end of 2003). Of the eligible 11,231 participants, 208 prevalent stroke (35 were haemorrhagic, 117 were ischaemic, and 56 were of unknown origin), and 1,508 without dose information were excluded. The final sample includes 9,515 AHS participants.

Radiation Dosimetry

Estimation of radiation dose exposure for each individual was based on an updated dosimetry system**(DS02)** that takes into account biases arising from errors in calculated doses, physical locations, and organ shielding at the time of bombing.[14] For all analyses, weighted colon doses were used in units of gray (Gy), where the dose for an individual corresponds to the total exposure in γ rays + 10× the smaller neutron dose.[14] **Colon dose was selected a priori because of broad (systemic) cardiovascular processes that are often associated with stroke.**

This includes the major system-wide precursors of hypertension, cigarette smoking, total cholesterol, diabetes and body mass. Colon dose was also used in an earlier study of circulatory disease in the RERF.[12]

Stroke Ascertainment

Possible stroke events and the underlying cause of death were coded according to International Classification of Disease **(ICD) in the RERF database. The ICD codes of stroke-related disease are 330-332, 334, 352, and 435 (ICD-7), 333, 430-434, 436, and 438 (ICD-8), 430, 431, and 433-438 (ICD-9), and G45, I60, I61, I63-66, and I69 (exclude I698) (ICD-10).** Virtually all deaths are assumed accounted for based on access to a comprehensive nationwide registration of deaths in Japan. The number of missed cases of nonfatal strokes in subjects who remained alive at the close of follow-up (2003) is unknown, although with high participation across repeated AHS examinations, it is thought to be small. There is no indication of bias in the indexing of stroke by radiation exposure. All data (health exams, death certificates, and autopsy reports when available) were reviewed to confirm the first-ever stroke. Stroke was defined as an acute-onset focal neurological deficit of vascular etiology, persisting for at least 24 hours. Stroke subtypes (ischaemic and haemorrhagic events) were categorized based on established criteria that included clinical features, neuroimaging and noninvasive vascular studies, and other laboratory criteria according to the definitions of typical/atypical stroke symptoms in the World Health Organization (WHO) Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Projects.[15] Ischaemic stroke was diagnosed if there was a focal neurological deficit in the absence of haemorrhage based on neuroimaging, when the neuroimage showed an ischaemic infarct that correlated with the clinical deficit, or an ischaemic infarct was documented at autopsy. Not all diagnoses of stroke were based on neuroimaging studies. Among **all strokes, 49.0% were based on death certificates alone (50.2% for haemorrhagic stroke and 49.3%**

for ischaemic stroke).

Baseline Examination and Questionnaires

Baseline concomitant data included the age when follow-up began, systolic blood pressure (SBP), total cholesterol (T-CHO), body mass index (BMI), diabetes, smoking, and alcohol intake**. A priori selection of these risk factors was based on a perceived need to consider traditional stroke risk factors,to include adjustments[16-18] that were made in an earlier report from the RERF,[12] and to consider possible sources of confounding due to documented relationships between radiation exposure, SBP,[19] and T-CHO.[20] Except for smoking and alcohol intake, the concomitant data were collected at clinical examinations that were given in 1980. In the event that an examination cycle failed to coincide with 1980, information from the most recent examination was used (with 5 years). Measurement of nonfasting T-CHO is described elsewhere.[20] Sitting SBP was measured in the left arm. BMI was defined as weight (kg) divided by height squared (m). A diagnosis of diabetes was based on a physician diagnosis or the use of medications for diabetes.**

Data on smoking and alcohol intake were collected from mail surveys that were administered from 1978 to 1980. In the absence of such data, information was taken from **direct interviews** in 1965. Smoking status was defined as never, past, and current smoker. Alcohol intake was defined according to typical Japanese consumption strata in units of ethanol as nondrinker, light drinker (<34 g/d), and heavy drinker (≥34 g/d).[21] **For the sample with interview data from 1965 and the mail survey in 1978-1980, smoking status was unchanged in over 87% of the study participants. In contrast, patterns of alcohol intake are more variable. The correlation between the repeated alcohol measures is 0.24, although it is highly significant (p<0.001).**

Statistical methods

Crude and age-adjusted incidence of haemorrhagic and ischaemic stroke in person-years of follow-up were estimated across common ranges of radiation dose based on standard analysis of covariance methods.[22] **Further description of the procedure used in the calculation of person-years is given elsewhere, along with its close relationship with a Cox proportional hazards regression model.[23] Similar methods are also useful for providing age-adjusted** percents and average levels of the confounding risk factors **across the radiation strata.[19]**

The primary method for testing for an independent effect of radiation on the risk of each stroke subtype **is based on Cox** proportional hazards regression models **where** radiation dose **is** modeled as a continuous predictor variable. **Although the number of strokes is often small (particularly haemorrhagic events), we found no evidence for a significant departure from the assumption of proportionality. This assumption is further relaxed by adjusting for age based on the use of attained age as the time scale in the nonparametric part of the hazard model while radiation dose and the concomitant risk factors were modeled as covariates in the parametric part. [24] Concomitant risk factors included** SBP and the other risk factors. Nonlinear relationships between radiation dose and the stroke risk were also considered, including a threshold analysis. For the latter, dose was modeled as $(D-\delta) \times I_{\delta}(D)$ where D is a radiation dose, δ is a threshold, and $I_{\delta}(D) = 1$ when $D \geq \delta$ and 0 otherwise. The dose that minimizes $-2 \times$ the log likelihood provides a point estimate for δ. A 95% confidence interval for δ consists of upper and lower threshold values for which $-2 \times$ the log likelihood differs from the minimum value by 3.84 (the 95th percentile from a χ^2 distribution with 1 degree of freedom). If the lower threshold value is >0 Gy, then a dose threshold is assumed to exist with 95% confidence. Although primary tests of significance were based on radiation dose being modeled as a continuous risk factor, patterns of association are also described through the use of indicator variables that allow for the estimation of the relative hazard of stroke (and 95 percent confidence

intervals) between radiation dose strata ≥0.05 Gy versus doses considered to be small (<0.05 Gy). All reported p-values were based on two-sided tests of significance. Statistical modeling and testing were based on the use of SAS software (version 9.2, SAS Institute Inc., Cary, NC, USA).

Results

Radiation exposure and study characteristics

Table 1 provides the distribution of radiation exposures, average baseline ages, and age-adjusted means and percent characteristics for the sample of men and women who were available for follow-up. The average radiation dose **(± standard deviation) for men is 0.41 ± 0.62 and for women 0.36 ± 0.55 (p<0.001).** For men, 15.3% were exposed to radiation doses ≥1 Gy, while 46.5% were exposed to doses <0.05 Gy. For women, corresponding percents were 11.6 and 44.6%.

After adjusting for differences in baseline age across the ranges of radiation exposure, BMI declined **(although modestly)** with increased radiation exposure in men (p=0.016) but not in women. For women, higher radiation doses were more likely associated with elevated SBP (p=0.013). A similar pattern was absent in men. While not significant, T-CHO levels were highest in women who had the greatest dose exposures. For the remaining data, associations with radiation were absent.

Age-adjusted incidence of stroke by radiation exposure

During the course of follow-up, there were 235 haemorrhagic and 607 ischaemic strokes (14.0 and 36.1/10,000 person-years, respectively). The average age at the time of a stroke was 73.2 years (range: 43 - 98 years) for those that were haemorrhagic and 77.0 years (range: 48 - 100 years) for those that were ischaemic. The average follow-up time before stroke occurrence was 11.1 years (range: 3 months - 23 years) for haemorrhagic events and 11.5 years (range: 4 days - 23 years) for ischaemic events. There were an additional 84 strokes that were of unknown subtype.

Table 2 provides further details on stroke incidence that was identified according to radiation exposure at the time of bombing. For men, after adjusting for age, the incidence of

haemorrhagic stroke rose consistently from 12.2 to 25.2/10,000 person years as radiation exposure increased from <0.05 to ≥2 Gy (p=0.006). Risk of haemorrhagic stroke continued to rise with increasing doses <1 Gy (p=0.006). For women, differences in the risk of haemorrhagic events were modest for doses <2 Gy but more than tripled when doses went higher (p=0.002). There were no significant relationships between radiation and the risk of ischaemic events for either sex (table 2). Relationships to total stroke were largely determined through associations with haemorrhagic events. There were no relationships between radiation and the incidence of strokes that were of unknown origin.

Risk factor adjusted associations between haemorrhagic stroke and radiation exposure

The association between radiation and haemorrhagic events was further examined after adjustment for age, SBP, BMI, diabetes, T-CHO, cigarette smoking, alcohol drinking, and city (table 3). For men, the association was diminished but remained significant (p=0.009). For those exposed to the highest amounts of radiation (≥2 Gy), there was a 2.5-fold excess risk of stroke as compared to doses that were <0.05 Gy (29.1 versus 11.6/10,000 person-years). Risk of haemorrhagic stroke also rose with increasing doses <1 Gy (p=0.004) suggesting that the dose-response relationship in men is not entirely attributed to the excess of haemorrhagic events that were observed in the highest ranges of radiation.

For women, while a dose-response relationship across the entire range of radiation and the risk of haemorrhagic events was absent, evidence in table 2 suggests that there may exist a threshold effect. Further analyses identified a significant dose threshold at 1.3 Gy (95% confidence interval, 0.5 - 2.3 Gy) where a change occurs in the association between radiation and the risk of haemorrhagic stroke. Below the threshold, risk was unrelated to radiation, while above the threshold, it increased with rising dose. This can better be seen in table 3 for dose strata <0.05, 0.05 to <1.3, 1.3 to <2.2, and ≥2.2 Gy. For doses <1.3 Gy, differences in

haemorrhagic risk were modest (14.2 and 13.0/10,000 person-years) while it increased to 20.3/10,000 person-years for doses that ranged from 1.3 to <2.2 Gy and to 48.6/10,000 person-years for doses that were higher. For men, a dose threshold was absent. In both sexes, findings do not appear to be influenced by events that occur at an early age (<55 years).

Discussion

Findings suggest that exposure to increasing radiation doses among atomic-bomb survivors beyond a threshold of 1.3 Gy is associated with an increase in the future risk of haemorrhagic stroke in women. While 1.3 Gy is only a point estimate, the lower 95% confidence limit (0.5-2.3 Gy), further suggests that there is more than 95% confidence that the true threshold is 0.5 Gy or higher. For men, the incidence of haemorrhagic stroke rose consistently with increasing exposure levels without evidence for a threshold. Even within the dose range <1 Gy, the dose-response observed in men persisted. **The 35-year period from the time of the atomic bombing in Hiroshima and Nagasaki to the beginning of stroke follow-up in 1980** may be especially meaningful **with regard to the increased** use of radiotherapy **at** younger ages **and the increased opportunity for** a stroke to develop **in later life**.[2]

Patterns of association persisted for the period that predated the 1980 baseline, during a time when enrollment in the AHS continued to be ongoing. For the 1980 baseline used in the current report, more than 97% of the AHS participants had been enrolled. The 1980 baseline also provides a uniform beginning with a fixed lag time since the bombing of Hiroshima and Nagasaki. More complete data from clinical examinations and a recently conducted mail survey were also available. Although many stroke diagnoses were based on death certificates alone (about 50%), confounding due to changes in the diagnosis of stroke through the advent of neuroimaging in the late 1970s was also thought to be minimized. With regard to the diagnostic uncertainty that is common in any large-scale study, best attempts were made for the proper classification of fatal and nonfatal strokes with the opportunity for adjudication among the study investigators. In the absence of neuroimaging, however, diagnostic limitations are difficult to avoid. In spite of evidence from Japanese samples that suggest that errors in stroke classification could be small,[25 26] subtle distinctions between primary intracerebral haemorrhage and

ischaemic events can still exist when neuroimaging is available.

There is also evidence of an excess risk of circulatory disease at low and moderate doses (<5Gy) in Japanese atomic-bomb survivors in the Life Span Study cohort [12] where follow-up began in 1950. Although there were no clinical examinations in the LSS, there was a 9% excess risk of death due to all strokes combined per unit Gy (p=0.02**). The 5% excess relative risk (95%CI: -6 to 17) that was observed for cerebral haemorrhage was indistinguishable from a 4% excess relative risk (-10 to 20) for cerebral infarction.**[12] Corroborating evidence also appears elsewhere, [5 10] while reports of uncertainty in the association between radiation and stroke are common,[11 27] most likely due to the extreme difficulties in quantifying radiation exposure in studies that often rely on limited record keeping and historical recall.

Given that the association between radiation and the risk of stroke is plausible, an explanation for the association is far from clear. **At high doses (>10Gy), there is well-established evidence from radiotherapy patients of direct damage in circulatory systems, predominantly the consequence of excessive cell killing and the associated response to cell damage. In contrast, epidemiologic studies suggest that the mechanisms associated with low and moderate-dose ionizing radiation (<5Gy) are different**.[16-18] More direct mechanistic derangements that might explain an association with haemorrhagic stroke include fibrinoid necrosis of the small arteries and arteriole, a common underlying cause for intracerebral haemorrhage due to hypertension **in murine brain** [28] **or arteriovenous malformations in humans.**[29] Fibrinoid necrosis in vessels is also preceded by proinflammatory cytokines and observed in late cerebral radionecrosis at radiotherapy doses less than 0.05 Gy.[30] Elevated blood pressure,[19] hypertension,[31] and inflammation (C-reactive protein and interleukin-6) among the atomic-bomb survivors [32 33] might further promote a fibrinoid necrosis link with haemorrhagic stroke. Hypertension has a greater impact on haemorrhagic stroke incidence than cerebral infarction.[34] In the absence of a clear explanation for the reported findings, further

study of subclinical arteriosclerosis or biological evidences among the AHS may provide additional insight into the role that radiation has on promoting stroke and its subtypes.

In the current report, several notable limitations need to be addressed. Whether findings apply to other ethnicities is unknown. Genetic susceptibilities may be different. The number of haemorrhagic events is also limited and **too insufficient to allow for a careful assessment of the effect of radiation on the risk of stroke between risk factor strata**. In spite of the limitations, findings from the AHS warrant consideration and further study. Attractive features include the high rate of participation between examination cycles and free access to standardized medical care and evaluation. Measurement of radiation exposure also adheres to a rigorous system of quantification.[14] Among large cohort studies, the dosimetry in the AHS is unusually precise. In conclusion, the risk of haemorrhagic stroke increases with rising radiation exposure for both sexes. In men, it seems to occur across the full range of radiation exposures, while in women, the risk becomes apparent when doses exceed a threshold at about 1.3 Gy. Given the observed latency between radiation exposure and haemorrhagic stroke in the current study, a consequence of the expanded use of radiotherapy in younger individuals[2] could be an increased opportunity for the development of adverse outcomes in later life.

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Author Contributions: IT contributed to the study design, interpreted the data, and took the lead in writing the paper. RDA conducted the statistical analyses, interpreted the data, and made substantial contributions to drafting the methods and results sections of the paper. TO and TT played a lead role in data acquisition. They also contributed to the study design and in writing the paper. KO, MA, and SF had important roles in data interpretation, in writing and reviewing the paper and provided essential oversight and supervision of the study design. KK and MM assisted in writing and supervision of the final draft of the paper. Their oversight of the final product for verification of the study findings was essential. Credit for all authors is based on 1) substantial contributions to conception and design (IT, RDA, TO, TT, KO, MA, SF), acquisition of data (TO, TT), or analysis and interpretation of data (IT, RDA, TO, TT, KO, MA, SF, KK, MM); 2) drafting the article or revising it critically for important intellectual content (all authors); and 3) final approval of the version to be published (all authors). Each author participated sufficiently in the work and accepts public responsibility for appropriate portions of the content in which they have an expertise. All authors critically revised the manuscript for important intellectual content.

Dare sharing statement There is no additional data available.

References

1. Mettler FA, Jr., Bhargavan M, Faulkner K, et al. Radiologic and nuclear medicine studies in the United States and worldwide: frequency, radiation dose, and comparison with other radiation sources--1950-2007. *Radiology* 2009;253:520-31.
2. Bhargavan M. Trends in the utilization of medical procedures that use ionizing radiation. *Health Phys* 2008;95:612-27.
3. Mettler FA, Jr., Thomadsen BR, Bhargavan M, et al. Medical radiation exposure in the U.S. in 2006: preliminary results. *Health Phys* 2008;95:502-7.
4. Dorresteijn LD, Kappelle AC, Scholz NM, et al. Increased carotid wall thickening after radiotherapy on the neck. *Eur J Cancer* 2005;41:1026-30.
5. Carr ZA, Land CE, Kleinerman RA, et al. Coronary heart disease after radiotherapy for peptic ulcer disease. *Int J Radiat Oncol Biol Phys* 2005;61:842-50.
6. Howe GR, Zablotska LB, Fix JJ, et al. Analysis of the mortality experience amongst U.S. nuclear power industry workers after chronic low-dose exposure to ionizing radiation. *Radiat Res* 2004;162:517-26.
7. Ivanov VK. Late cancer and noncancer risks among Chernobyl emergency workers of Russia. *Health Phys* 2007;93:470-9.
8. Scott AS, Parr LA, Johnstone PA. Risk of cerebrovascular events after neck and supraclavicular radiotherapy: a systematic review. *Radiother Oncol* 2009;90:163-5.
9. Hauptmann M, Mohan AK, Doody MM, et al. Mortality from diseases of the circulatory system in radiologic technologists in the United States. *Am J Epidemiol* 2003;157:239-48.
10. McGeoghegan D, Binks K, Gillies M, et al. The non-cancer mortality experience of male workers at British Nuclear Fuels plc, 1946-2005. *Int J Epidemiol* 2008;37:506-18.
11. Muirhead CR, O'Hagan JA, Haylock RG, et al. Mortality and cancer incidence following occupational radiation exposure: third analysis of the National Registry for Radiation Workers. *Br J Cancer* 2009;100:206-12.
12. Shimizu Y, Kodama K, Nishi N, et al. Radiation exposure and circulatory disease risk: Hiroshima and Nagasaki atomic bomb survivor data, 1950-2003. *BMJ* 2010;340:b5349.
13. Preston DL, Pierce DA, Shimizu Y, et al. Effect of recent changes in atomic bomb survivor dosimetry on cancer mortality risk estimates. *Radiat Res* 2004;162:377-89.
14. Cullings HM, Fujita S, Funamoto S, et al. Dose estimation for atomic bomb survivor studies: its evolution and present status. *Radiat Res* 2006;166:219-54.
15. Asplund K, Bonita R, Kuulasmaa K, et al. Multinational comparisons of stroke epidemiology. Evaluation of case ascertainment in the WHO MONICA Stroke Study. World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease. *Stroke* 1995;26:355-60.
16. Little MP, Tawn EJ, Tzoulaki I, et al. Review and meta-analysis of epidemiological associations between low/moderate doses of ionizing radiation and circulatory disease risks, and their possible mechanisms. Radiat Environ Biophys 2010;49:139-53.
17. Little MP, Tawn EJ, Tzoulaki I, et al. A systematic review of epidemiological associations between low and moderate doses of ionizing radiation and late cardiovascular effects, and their possible mechanisms. Radiat Res 2008;169:99-109.
18. UNSCEAR. United Nations Scientific Committee on the Effects of Atomic Radiation 2006 Report to the General Assembly with Scientific Annexes. Sources and Effects

of Ionizing Radiation. Volume I. Annex B: Epidemiological evaluation of cardiovascular disease and other non-cancer diseases following radiation exposure. .

19. Sasaki H, Wong FL, Yamada M, et al. The effects of aging and radiation exposure on blood pressure levels of atomic bomb survivors. *J Clin Epidemiol* 2002;55:974-81.
20. Yamada M, Wong FL, Kodama K, et al. Longitudinal trends in total serum cholesterol levels in a Japanese cohort, 1958-1986. *J Clin Epidemiol* 1997;50:425-34.
21. Kiyohara Y, Kato I, Iwamoto H, et al. The impact of alcohol and hypertension on stroke incidence in a general Japanese population. The Hisayama Study. *Stroke* 1995;26:368-72.
22. Lane PW, Nelder JA. Analysis of covariance and standardization as instances of prediction. *Biometrics* 1982;38:613-21.
23. Abbott RD. Logistic regression in survival analysis. *Am J Epidemiol* 1985;121:465-71.
24. Korn EL, Graubard BI, Midthune D. Time-to-event analysis of longitudinal follow-up of a survey: choice of the time-scale. *Am J Epidemiol* 1997;145:72-80.
25. Tanaka H, Ueda Y, Hayashi M, et al. Risk factors for cerebral hemorrhage and cerebral infarction in a Japanese rural community. *Stroke* 1982;13:62-73.
26. Tanaka H, Ueda Y, Date C, et al. Incidence of stroke in Shibata, Japan: 1976-1978. *Stroke* 1981;12:460-6.
27. Vrijheid M, Cardis E, Ashmore P, et al. Mortality from diseases other than cancer following low doses of ionizing radiation: results from the 15-Country Study of nuclear industry workers. *Int J Epidemiol* 2007;36:1126-35.
28. Daigle JL, Hong JH, Chiang CS, et al. The role of tumor necrosis factor signaling pathways in the response of murine brain to irradiation. *Cancer Res* 2001;61:8859-65.
29. da Costa L, Wallace MC, Ter Brugge KG, et al. The natural history and predictive features of hemorrhage from brain arteriovenous malformations. *Stroke* 2009;40:100-5.
30. Xie CH, Zhang MS, Zhou YF, et al. Chinese medicine Angelica sinensis suppresses radiation-induced expression of TNF-alpha and TGF-beta1 in mice. *Oncol Rep* 2006;15:1429-36.
31. Yamada M, Wong FL, Fujiwara S, et al. Noncancer disease incidence in atomic bomb survivors, 1958-1998. *Radiat Res* 2004;161:622-32.
32. Hayashi T, Kusunoki Y, Hakoda M, et al. Radiation dose-dependent increases in inflammatory response markers in A-bomb survivors. *Int J Radiat Biol* 2003;79:129-36.
33. Neriishi K, Nakashima E, Delongchamp RR. Persistent subclinical inflammation among A-bomb survivors. *Int J Radiat Biol* 2001;77:475-82.
34. Takahashi I, Geyer SM, Nishi N, et al. Lifetime risk of stroke and impact of hypertension: estimates from the adult health study in Hiroshima and Nagasaki. *Hypertens Res* 2011;34:649-54.

Table 1. Distribution of radiation exposures, average baseline age, and age-adjusted means and percent characteristics for the sample of men and women available for stroke follow-up

Characteristic	Radiation exposure (Gy)			
	<0.05	0.05 to <1	1 to <2	≥2
Men (sample size)	1539	1266	376	130
Percent of sample	46.5	38.2	11.4	3.9
Baseline age* (y)	57 ±13	57 ±13	54 ±13	51 ±11
SBP (mmHg)	135 ±24	135 ±23	133 ± 20	136 ± 19
T-CHO (mmol/L)	4.53 ± 0.88	4.60 ± 0.85	4.65 ± 0.96	4.55 ± 0.96
BMI† (kg/m^2)	21.9 ± 3.0	21.8± 3.0	21.7 ± 2.9	21.5 ± 3.0
Diabetes (%)	12.6	14.6	12.5	15.5
In Nagasaki at exposure	40.8	31.1	41.2	30.2
Smoking status (%)				
Past	21.6	21.0	22.7	16.8
Current	66.6	65.6	64.2	68.5
Alcohol intake (%)				
Light (<34 g/day)	44.3	42.0	42.9	44.1
Heavy (≥34 g/day)	38.3	38.3	41.2	38.4
Women (sample size)	2765	2720	531	188
Percent of sample	44.6	43.8	8.6	3.0
Baseline age* (y)	59 ±13	60 ±13	58 ±12	56 ±12
SBP‡ (mmHg)	134 ±26	135 ±26	136 ±25	137 ±26
T-CHO§ (mmol/L)	4.94 ± 0.91	5.02 ± 0.96	5.09 ± 1.01	5.15 ± 0.93
BMI (kg/m^2)	22.8 ±3.6	22.9 ±3.5	23.0 ± 3.5	22.3 ± 3.2
Diabetes (%)	6.7	8.4	8.0	7.2
In Nagasaki at exposure‖	31.0	26.5	35.7	25.8
Smoking status (%)				
Past	5.1	4.3	5.3	7.2
Current	10.6	14.7	13.8	13.1
Alcohol intake (%)				
Light (<34 g/day)	20.7	21.3	21.3	16.2
Heavy (≥34 g/day)	0.8	1.9	1.3	0.0

Continuous variables are reported as means ± standard deviations. The remaining variables are reported as percents.

*Significant decline with radiation exposure (p<0.001)

† Significant decline with radiation exposure (p=0.016)

‡ Significant increase with radiation exposure (p=0.013)

§ Significant increase with radiation exposure (p<0.001)

‖Significant increase with radiation exposure (p=0.002)

Abbreviations: SBP, systolic blood pressure; BMI, body mass index; T-CHO, total cholesterol

Table 2. Age-adjusted stroke incidence by radiation exposure.

Radiation exposure (Gy)	Sample size	Haemorrhagic		Ischaemic		Total stroke	
		Events	Incidence*	Events	Incidence*	Events	Incidence*
			Men				
<0.05	1539	33	12.2	112	40.5	154	56.1
0.05 to <1	1266	37	17.6	81	38.5	132	62.7
1 to <2	376	13	21.4	20	34.9	36	61.9
≥2	130	5	25.2	8	46.3	13	72.4
p-value†			0.006‡		0.788		0.202
Overall	3311	88	15.7	221	39.4	335	59.7
			Women				
<0.05	2765	66	13.1	173	34.4	262	52.0
0.05 to <1	2720	63	12.4	174	33.9	264	51.6
1 to <2	531	8	9.3	33	39.8	46	54.9
≥2	188	10	41.9§	6	27.9	19	85.7‖
p-value			0.098		0.930		0.155
Overall	6204	147	13.1	386	34.4	591	52.7

*Incidence rate per 10,000 person-years

†The p-value is a test for trend with dose modeled as a continuous variable.

‡For men, risk of haemorrhagic stroke continued to rise with increasing doses <1 Gy (p=0.006).

§Risk of haemorrhagic stroke in women is higher for doses ≥2 Gy versus lower doses (p=0.002).

‖Risk of total stroke in women is higher for doses ≥2 Gy versus lower doses (p=0.027).

Table 3. Risk factor adjusted incidence and relative hazards of haemorrhagic stroke by radiation exposure

Radiation exposure (Gy)	Incidence	Relative hazard (95% CI)
	Men	
<0.05	11.6	reference
0.05 to <1	17.7	1.5 (0.8, 2.7)
1 to <2	20.2	1.7 (0.7, 4.1)
≥2	29.1	2.5 (0.8, 7.3)
p-value†	0.009‡	
	Women	
<0.05	14.2	reference
0.05 to <1.3	13.0	0.9 (0.6, 1.4)
1.3 to <2.2	20.3	1.4 (0.6, 3.7)
≥2.2	48.6	3.5 (1.4, 9.0)
p-value	0.002	

Incidence rate per 10,000 person-years and relative hazards are adjusted for age, systolic blood pressure, body mass index, diabetes, total cholesterol, cigarette smoking, alcohol drinking, and city.

†The p-value is a test for trend with dose modeled as a continuous variable. For women, a dose threshold model is used with a dose threshold at 1.3 Gy (95% CI, 0.5– 2.3 Gy).

‡For men, risk of haemorrhagic stroke continued to rise with increasing doses <1 Gy (p=0.004).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3, 4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	1, 4, 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-9
Bias	9	Describe any efforts to address potential sources of bias	7, 15
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9-11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9-11
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	7-9
		(*d*) If applicable, explain how loss to follow-up was addressed	7-9
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12 and Table1
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) Summarise follow-up time (eg, average and total amount)	12
Outcome data	15*	Report numbers of outcome events or summary measures over time	12 and Table 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-14, and Table 2-3
		(*b*) Report category boundaries when continuous variables were categorized	13 and Tables 1-3
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Table2
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	2, 15
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.