

Departmental Overview

The Department of Clinical Studies conducts health-examination and research programs including the Adult Health Study (AHS) and the F₁ (children of the atomic bomb survivors) Offspring Clinical Study (FOCS). The objectives of the AHS are 1) to monitor development of multifactorial diseases based on clinical follow-up, 2) to identify diseases that are associated with radiation exposure, and 3) to facilitate examining potential underlying mechanisms of increased risks for diseases in collaboration with the other departments at RERF as well as outside investigators. The objectives of the FOCS are 1) to determine potential heritable effects of radiation exposure based on clinical follow-up, and 2) to facilitate multidisciplinary studies to characterize effects and underlying mechanisms in collaboration with Departments of Epidemiology, Statistics, and Molecular Biosciences. These studies provide the opportunity for a number of specific investigations within RERF, and in collaboration with external investigators, to be conducted examining a variety of health outcomes and the understanding of mechanisms.

The AHS biennial health examinations were initiated in 1958 and continue today. The AHS cohort consists of a subcohort of the Life Span Study (LSS). This consists of A-bomb survivors of all ages at exposure, including those exposed *in utero*. These health examinations represent the only point of regular direct contact with the survivors and provide health benefits to that population through early disease detection. Such examinations function as the principal source of biological materials that make possible a wide variety of valuable studies by numerous RERF departments and outside investigators. Sera, blood cells, plasma, and urine have been longitudinally collected from the AHS participants and stored since 1969, 1990, and 1999, respectively based on newly obtained informed consent. The AHS program has greatly contributed to RERF's mission of 1) assessing noncancer disease risks from radiation, 2) determining radiation effects on physiological or biochemical abnormalities and correlating this information with other life experiences and modes and patterns of disease, and 3) elucidating mechanisms of radiation effects on cancer and noncancer diseases using stored biosamples and clinical, physiological, and epidemiological information obtained through the health examinations.

The AHS continually increases in importance as a result of the accumulation of a large body of clinical and epidemiological data from the 29 rounds of biennial health examinations carried out to date. The AHS has provided the strongest available data to analyze radiation-related increases in morbidity at low-to-moderate doses for noncancer diseases, such as cardiovascular disease (CVD), hyperparathyroidism, thyroid disease, chronic hepatitis B

virus infection, and cataracts, plus subclinical risk indicators and conditions such as inflammation or insulin resistance.

In 2002, the Department of Clinical Studies began the FOCS in which examinations have been conducted for about 12,000 individuals to analyze the potential heritable effect(s) of A-bomb exposure on polygenic, multifactorial diseases (e.g., diabetes, hypertension, dyslipidemia, coronary heart disease, and stroke) based on prevalence data obtained from 2002 to 2006. However, owing to the young age of the F₁ group (mean age of about 49 years at that time), most of their disease experience was still ahead. Therefore we converted the sample to a cohort for prospective follow-up and started health examinations every 4 years in November 2010.

Epidemiological studies of health effects in Fukushima emergency workers (Nuclear Emergency Workers Study: NEWS) were initiated in 2014. An office of the NEWS was placed within the Department of Clinical Studies, Hiroshima in 2015. Various experiences derived from health-examination programs of the AHS or the F₁ Clinical Study have been used in the conduct of the longitudinal health study of NEWS.

FY2017 Achievements***Radiation and Cancer***

Three nested case-controlled studies have been conducted based on use of stored sera or blood cells obtained from AHS participants to investigate whether risk factors such as infectious agents, hormones, or lifestyle-related factors modify each radiation risk. We worked on gastric and liver cancer studies as a PI and breast cancer study as a collaborator.

[Gastric cancer study]

- Continued reanalysis of the joint effects of radiation and chronic gastritis upon gastric cancer risk by pathological types. The results indicated that radiation exposure is associated with increased risk of diffuse-type noncardia gastric cancer without chronic atrophic gastritis.

[Liver cancer studies]

- Recent results from RERF showed that HBV and HCV infection and radiation exposure are associated independently with increased risk of HCC, and that radiation exposure was a significant risk factor for non-B, non-C HCC with no apparent confounding by alcohol consumption, smoking habit, or BMI. We are now conducting a study the objective of which is to examine whether chronic inflammation due to radiation exposure is involved in the development of HCC through insulin resistance or liver fibrosis, regardless of HBV/HCV infection and lifestyle-related factors. We have worked on a statistical model for inferring the latent factors (inflammation, insulin resistance, liver fibrosis, etc.) based on values of biomarkers in collaboration with the Statistics and Epidemiology Departments.
- Multidisciplinary program projects on radiation and liver cancer have been proposed by involving researchers from the Departments of Statistics, Molecular Biosciences, and Epidemiology within the cancer research cluster (i.e., Projects related to mechanisms of radiation risk in HCC associated with HBV/HCV, Projects related to mechanisms of radiation-associated non-B/non-C HCC, Projects related to joint effects of radiation and lifestyle factors including effect modification, Projects related to methodological issues). Overall objectives are to clarify and quantify mechanisms of radiation risk for HCC in order to 1) better understand the underlying biological and clinical aspects, 2) obtain more-precise estimates of risk, and 3) be able to apportion probability of causation in radiation-associated HCC.

[Pathogenesis of Myelodysplastic Syndrome (MDS)]

- Radiation is one of the causes of the development of hematological malignancies. A-bomb survivors have a high risk of hematological malignancies, even 50 years after exposure, such as acute myeloid leukemia (AML) and MDS. Recent genome analyses of these diseases have demonstrated that most of samples contain several gene mutations. Objectives are to determine mutated clones before clinical diagnosis of MDS and to explore how it differs by exposed radiation dose. We have a plan to detect mutations in AHS participants who developed MDS using next-generation genome analysis technology. This study will answer the very important question about how radiation-induced myeloid malignancies develop. Furthermore, we will be able to obtain new knowledge of mechanisms of radiation-induced myeloid malignancies.

Radiation and Noncancer Condition: Cataract

Radiation effects on posterior sub-capsular opacity have been well documented among A-bomb survivors and other exposed population. With regard to radiation effects on cortical/nuclear opacities, however, evidence appears to be insufficient and study results are inconsistent. Therefore, we are conducting a new ophthalmological study to obtain lens images by 3 devices; slit-lamp, retro-illumination camera, and Scheimpflug camera with standardized method.

- Ophthalmologic examinations for our cataract study using these devices were initiated in Hiroshima and Nagasaki in collaboration with ophthalmologists in Hiroshima and Nagasaki Universities in April 2016. Supervision for this study is made by a cataract specialist and scoring of cataract severity will be made by the same person using photographed images. Approximately 600 participants have been examined by ophthalmologists and their lens images were obtained.

Radiation and Noncancer Condition: Cardiovascular Disease (CVD)

It has been recognized since the 1960s that the heart may be damaged by substantial doses of radiation (> 30 Gy), such as doses used during mantle radiotherapy for Hodgkin lymphoma. With regard to lower dose radiation, epidemiological data are insufficient and biologically plausible mechanisms are lacking, although there are several theories which may be applied to lower dose exposure, including microvasculature effects, oxidation, inflammation, and mutation theories. Previous results from the LSS and AHS indicated the association between radiation exposure and CVD mortality or incidence, but the diversity of disease subtypes and confounding risk factors related to CVD risk complicate the estimates of radiation effects. We are conducting studies the objectives of which are to examine in detail the association between radiation exposure and arteriosclerosis, heart disease, chronic kidney disease (CKD), and atrial fibrillation (AF).

- Although the LSS study and other studies in the literature have recently identified radiation risks for CVD mortality or incidence at low-to-moderate doses, the etiological and pathogenic pathways are not well characterized. To better characterize these pathways, we have been examining physiological indices of arteriosclerosis and potential related biomarkers with radiation dose in the AHS. Pulse wave velocity, augmentation index, ankle-brachial blood pressure index (ABI), carotid intima-media wall thickness, and aortic calcification in thoracic and abdominal aorta were measured as physiological indices of arteriosclerosis. Multi-functional cytokines, possibly related to radiation-induced atherogenesis also have been measured. The complex pathways involving these indices and biomarkers are now being assessed using structural equation models. This study showed no clear association between prevalence of peripheral artery disease diagnosed by ABI and radiation exposure in the AHS.
- The LSS and certain other studies have suggested that heart failure and valvular disease, in addition to ischemic heart disease, are associated with radiation exposure. We have conducted the study using echocardiography and relevant biomarkers to obtain early indicators of these types of disease and to determine these disease risks among about 2,700 AHS participants.
- Continued analyses of the association of CKD and albuminuria with radiation dose.

- Continued analyses of the association between AF and radiation dose.
- In terms of radiation-associated CVD, previous AHS findings have consistently suggested dose effects on myocardial infarction, especially in females and in the high-dose group. In particular, the AHS study from 1968 to 1998 found a quadratic dose–response relationship for non-fatal myocardial infarction for people exposed before 40 years of age, indicating a risk primarily at higher doses. Recent LSS study have also found a statistically significant association for radiation exposure and mortality for hypertensive heart disease, rheumatic or valvular heart disease and heart failure, but not for ischemic heart disease (IHD), including myocardial infarction. Studies of CVD mortality include the uncertainties in the diagnoses listed on death certificates. To determine the association between radiation exposure and CVD incidence, we have conducted medical chart review to detect CVD with consistent diagnostic criteria during 1958 and 2011 in the AHS.

Radiation and Noncancer Condition: Others

We are conducting additional studies focusing on the possible association between radiation exposure and other noncancer diseases or condition at low-to-moderate doses as well. The LSS data have suggested radiation-related increase risks in mortality for noncancer diseases such as circulatory disease and digestive disease. The AHS data have also suggested that A-bomb radiation effects increased risks or positive dose response in incidence for noncancer diseases such as diabetes, thyroid disease, and chronic liver disease. To determine whether such noncancer diseases are associated with radiation exposure, we aim to examine dose response relationships for noncancer diseases or condition and to determine possible mechanisms using discovery approaches. These studies will be developed within the noncancer research cluster with clinical and epidemiological data and longitudinal biosamples.

- ABCC-RERF has conducted several dose-response studies regarding the association between radiation and diabetes among AHS participants, but the findings were inconsistent. To determine whether radiation exposure is associated with the development of diabetes between Hiroshima and Nagasaki, and to investigate whether any such association is influenced by the city of exposure and age at exposure, we have conducted medical chart review using standardized criteria to detect diabetes among 12,759 original AHS participants since 1958, a total of 1863 subjects had diabetes, as of December 2017. Medical chart review is underway.
- We investigated the association between thyroid dysfunction or autoimmunity and low-to-moderate doses radiation (*J Clin Endocrinol Metab*, 2017; 102:2516-24).
- The LSS and AHS data have shown that chronic liver disease and liver cirrhosis are related to radiation dose. Chronic liver disease sometimes progresses into liver cirrhosis and HCC, among individuals suffering from chronic type B or C liver disease and nonalcoholic steatohepatitis. To determine whether A-bomb radiation exposure has increased liver stiffness, which serves as a marker of liver fibrosis severity, and to investigate the possibility that liver fibrosis is involved in the development of atherosclerotic diseases by inducing insulin resistance, we examined the association between measurement of liver stiffness with the elastometer and other blood fibrosis markers. Subsequent studies will test whether radiation exposure increase such effects.
- The AHS and various studies of childhood radiotherapy have shown that the brain is

susceptible to radiation damage *in utero* and in early childhood. ABCC/RERF and other studies have documented early-life cognitive deficits in relation to *in utero* and childhood radiation exposures. We conducted the Neurocognitive Questionnaire (NCQ) survey, which is a self-reported measure of subjective neurocognitive function, among subjects at ages <13 years at the time of the bombings, to examine the effect of radiation exposure on cognitive function as it appears more than 65 years later. Regarding the effects of demographic factors on subjective neurocognitive function among non-exposed subjects in the background analysis, the analysis showed that older age and lower education were associated with more subjective neurocognitive complaints.

Genetic Effects

The initial examination of the longitudinal F₁ clinical cohort from 2002 to 2006 (the first round examination) provided no evidence for an increased prevalence of adult-onset multifactorial diseases due to parental radiation exposure, but the study subjects were still quite young. Definitive human data can only be obtained if a high-quality clinical study is continued until the subjects become elderly, when many multifactorial diseases develop. The objective of this study is to elucidate the effects of parental exposure to A-bomb radiation on the development of polygenic, multifactorial diseases and subclinical conditions among the F₁ offspring. Self-selection bias also tends to be minimized when prospective longitudinal data are obtained, because such data allow estimates of disease incidence. Thus far we have:

- Completed the second round examination of the longitudinal F₁ offspring clinical study (FOCS) that was initiated in November 2010 on a four-year cycle. 10,426 subjects participated and participation rate during this cycle was 79.6%. Continued conducting the third round examination.
- Conducted preliminary tabulation of the prevalence and incidence of individual multifactorial disease outcomes among about 10,000 FOCS participants between November 2010 and October 2015.
- Initiated efforts to develop an integrated program project in collaboration with Departments of Epidemiology, Molecular Biosciences, and Statistics.

Nuclear Emergency Workers Study (NEWS)

The study includes about 20,000 nuclear emergency workers who were involved in emergency operations after the Fukushima Daiichi nuclear power plant accident, during the period of March 14, to December 16, 2011, when dose limit for emergency workers was raised from 100 mSv to 250 mSv. The objective of this study is to clarify the long-term health effects of radiation on nuclear emergency workers. The project consists of mortality and cancer incidence studies, a clinical study, special clinical studies on thyroid cancer, cataracts, and psychological effects, and a radiobiological study. During 2017, we continued to set up a research scheme, establish a cohort, and recruit study participants. Thus far:

- A total of approximately 6,700 potential subjects agreed to participate in the study and a total of approximately 3,900 participants completed general health examination as of November 2017.
- Thyroid cancer and psychological studies were also conducted at the same time as general health examination at the cooperative medical institutions. Cataract studies will be initiated with the cooperation of members of the Japanese Society for Cataract Research

and the Japanese Ophthalmological Society in 2018.

- With regard to mortality studies, permission of the Ministry of Health, Labour and Welfare (MHLW) was obtained to use computerized vital statistics data in October 2017.
- Continued reconstruction of individual radiation dose: in addition to the tentative dose provided by the MHLW, corporate records related to emergency operations were obtained in September 2017.