

Departmental Overview

The Department of Clinical Studies conducts health-examination and research programs including the Adult Health Study (AHS) that is consisted of atomic bomb survivors and the F₁ (their children) Offspring Clinical Study (FOCS). The mission of the Department of Clinical Studies is to provide leadership in clinical medicine and facilitate multidisciplinary research using information and biosamples obtained from AHS and FOCS participants. 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 using biosamples in collaboration with not only the other departments at RERF but also outside investigators. The objectives of the FOCS are 1) to monitor development of multifactorial diseases based on clinical follow-up, 2) to elucidate association of parental radiation exposure with development of diseases in their offspring, and 3) 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 biosamples 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 30 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 health examinations have been conducted for about 12,000 individuals to analyze the potential

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heritable effect(s) of A-bomb exposure on polygenic, multifactorial diseases (e.g., diabetes, hypertension, hypercholesterolemia, angina pectoris, myocardial infarction 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 the second round examinations of the FOCS every 4 years in November 2010. We have almost completed the third round of examinations and started the fourth round in November 2018.

Epidemiological studies of health effects in Fukushima emergency workers (Nuclear Emergency Workers Study: NEWS) were initiated in 2014. The emergency workers health study office was placed within the Department of Clinical Studies, Hiroshima in 2015, and the first phase of the NEWS was conducted from FY2014 to FY2018. In the second phase, the National Institute of Occupational Safety and Health, Japan (JNIOSH) succeeded RERF as the leading research institute in June 2019. RERF has joined in the NEWS as a main collaborative institute, and the Department of Clinical Studies has cooperated in the clinical study. Various experiences derived from health-examination programs of the AHS or the FOCS have been used in the conduct of the longitudinal health study of NEWS.

FY2019 Clinical Studies Department Achievements

Radiation and Solid Cancer

Two nested case-control 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, or lifestyle-related factors modify each radiation risk. We worked on gastric and liver cancer studies in collaboration with not only the Statistics and Epidemiology Departments but also outside investigators.

[Gastric cancer study]

- Recent results from RERF demonstrated that chronic atrophic gastritis, current smoking, and H. pylori infection are significant risk factors for noncardia gastric cancer, but radiation risks were modified by smoking status and pathological types. We evaluated the modifying effects of chronic gastritis on radiation risk of noncardia gastric cancer by histological types by reanalyzing data from a nested case-control study conducted within AHS participants. The results indicated that radiation exposure is associated with increased risk of diffuse-type noncardia gastric cancer without chronic atrophic gastritis, and this association exists despite adjustment for H. Pylori infection and smoking habits. A paper about the results has been accepted for publication (Radiat Res, 2020; 194(2):180-7).

[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 habits, 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 successfully fit a structural regression model for inferring the latent factors (inflammation, insulin resistance, liver fibrosis, etc.) based on values of biomarkers. Based on that model, we have estimated odds ratios for association between latent factors and HCC. We have also established, through methodological research, that standard mediation analysis can be applied to counter-matched, nested case-control data without modification. A paper on that topic has been accepted for publication (Biometrical Journal, in press).
- Multidisciplinary Liver Cancer Program is being developed within Cancer Research Cluster to clarify immunologic and metabolic mechanisms of radiation-related liver cancer. The research program consist of the following projects such as 1) Identification of novel biomarkers for detection, mechanisms, and radiation sensitivity of HCC among atomic bomb survivors, 2) Immune cell alterations predicting viral or non-viral hepatitis progression to HCC, 3) Roles of hepatic stellate cells and macrophages in the course of liver carcinogenesis in X-irradiated mice, and 4) misclassification of primary liver cancer in the RERF tumor registry and study cohorts. This research program involves collaborative studies with researches from Fukushima Medical University, Nagasaki University, and Hiroshima University as well as within RERF. The research protocol of project 3) has been prepared.

Radiation and hematological malignancies

Two studies are being conducted based on use of stored blood samples of myelodysplastic syndrome cases in the AHS and autopsy samples of chronic myeloid leukemia cases in the LSS. These studies will be able to provide us with new knowledge regarding the mechanisms of radiation-induced myeloid malignancies. We worked on the hematological malignancy studies in collaboration with not only the Statistics and Epidemiology Departments but also outside investigators.

[Pathogenesis of Myelodysplastic Syndrome (MDS)]

- This project has been developed in collaboration with the Nagasaki University and the Kyoto University. 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, and that these mutations might be found before clinical diagnosis. We hypothesize that a hematopoietic progenitor or stem cell with a small number of gene mutation acquires additional gene mutations over time (more than several years) and causes hematological malignancies and that ionizing radiation increases the chance of such gene mutations occurring. We have initiated a study to detect mutations in serially stored blood samples of AHS participants who developed MDS using next-generation genome analysis technology. Objectives are to determine dynamics of mutated clones before clinical diagnosis of MDS and to explore how it differs by exposed radiation dose. This study will answer the very important question about how radiation-induced myeloid malignancies develop, which has never been tested.

[Chronic myeloid leukemia (CML) study]

- Leukemia is the only malignancy that developed shortly after the A-bomb, and it is widely recognized that radiation exposure can induce leukemia. A study of leukemia morphological classification (French-American British classification) among A-bomb survivors in 1950-1980 revealed that chronic myeloid leukemia (CML) most frequently developed during the period. CML is currently diagnosed based on the presence of the fusion gene BCR-ABL1. Here, we are conducting a preliminary study to assess whether A-bomb survivors with CML carry BCR-ABL1 using formalin-fixed paraffin-embedded (FFPE) samples of autopsied cases. Additionally, ABL1 mutation analysis, which is a common mutation in CML, will be performed. Based on the data, we plan to examine genomic alterations in CML and the other types of leukemia cases, including acute myeloid leukemia using more FFPE samples of autopsied cases in a future full-scale study.

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 in Kanazawa Medical University. Ophthalmologic examinations among 1,045 AHS subjects who were <15 years of age at the time of bombings (including 115 in-utero exposed subjects) were finished in March 2020 in Hiroshima and Nagasaki. After scoring of cataract severity using photographed images was completed by an ophthalmologist, a statistical analysis will be started in collaboration with the Statistics Department.

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 atherosclerosis, heart disease, chronic kidney disease (CKD), and atrial fibrillation (AF).

- Past studies have reported an association between radiation exposure and cardiovascular disease mortality/morbidity. To assess the mechanism, we measured a comprehensive set of indicators of atherosclerosis including ABI, IMT, augmentation index (AI), central systolic blood pressure (cSBP), brachial-ankle pulse wave velocity (baPWV), upstroke time (UT), and aortic calcification evaluated from chest and lumbar X-rays among 3,775 AHS participants in 2010-2014. The results are now under submission to an international journal.
- The LSS and outside RERF studies have suggested that heart failure and valvular disease, in addition to ischemic heart disease, are associated with radiation exposure. We are conducting studies with the objective of evaluating radiation effects on diastolic heart failure, one subtype of heart failure among the AHS aged 15 or less at the time of bombing. We have obtained early indicators of these types of disease, using echocardiography and relevant biomarkers to determine these disease risks among 2,889 AHS participants.
- Previous report from RERF showed that CKD diagnosed using only estimated glomerular filtration rate (eGFR) is significantly associated with radiation dose among Nagasaki AHS participants. Using both urinary albumin and eGFR, analyses of the association of CKD and albuminuria with radiation dose was continued for AHS participants in Hiroshima and Nagasaki. To improve the assessment of outcomes for future analyses, we have updated the detailed questionnaires to screen heart diseases and stroke in both Hiroshima and Nagasaki.

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 thyroid disease, diabetes, 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 Non-Cancer Research Cluster with clinical and epidemiological data and longitudinal biosamples.

- In previous AHS thyroid studies (1st cycle; conducted 2007-2011), an increased risk of thyroid nodules was observed, while risks of thyroid dysfunction and autoimmunity were not apparent. However, careful interpretations are needed because only limited data from cross-sectional studies are available. We also have a limitation in analyzing thyroid diseases in those exposed in utero because of a small cohort size. However, a longitudinal study may improve the statistical power for analyses. We planned thyroid examinations once every four years and has been conducting 2nd cycle of thyroid examination such as blood tests and ultrasonography in younger exposed AHS participants including those exposed in utero since 2018.
- 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 ATB, we conducted medical chart review using standardized criteria to detect diabetes in the AHS. Among 15,113 AHS participants including in-utero exposed survivors, a total of 2,521 diabetes cases were identified. The statistical analysis regarding the association between radiation dose and incidence of diabetes has been started.
- 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 is associated with increase in liver stiffness. Discussions with newly assigned statistician have started.
- 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 have been conducting studies the objective of which is to examine the prenatal and childhood effects of radiation exposure on cognitive function as it appears more than 65 years later by using the Cognitive Abilities Screening Instrument (CASI) and the Neurocognitive Questionnaire (NCQ). Regarding NCQ, latent factors related subjective neurocognitive complaints were identified by an exploratory factor analysis among non-exposed subjects. An article about the effects of demographic variables on subjective neurocognitive complaints was published (Int J Environ Res Public Health, 2019; 16(3):

421-30). The results showed that older age and lower education were associated with more subjective neurocognitive complaints. We are also analyzing the radiation effect on subjective neurocognitive complaints based on NCQ.

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 such as diabetes, hypertension, dyslipidemia, ischemic heart disease, and stroke, 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:

- Almost completed the third round examination of the F1 offspring clinical study (FOCS) that was initiated in November 2014 on a four-year cycle. 9,750 subjects participated and participation rate during this cycle was 74.4%. Continued conducting the third round examination and started the fourth round in November 2018.
- Initiated efforts to develop an integrated program project in collaboration with Departments of Epidemiology, Molecular Biosciences, and Statistics. Organized as an umbrella program project for investigation of genetic effects of atomic bomb radiation to the Genetic Research Cluster.

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 through 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 including general health examination and storage of biosamples, special clinical studies on thyroid cancer, cataracts, and psychological effects, a radiobiological study and reconstruction of individual radiation doses. Epidemiological studies of health effects in Fukushima emergency workers (Nuclear Emergency Workers Study: NEWS) were initiated in 2014. The emergency workers health study office was placed within the Department of Clinical Studies, Hiroshima in 2015, and the first phase of the NEWS was conducted from FY2014 to FY2018. In the second phase, the National Institute of Occupational Safety and Health, Japan (JNIOSH) succeeded RERF as the leading research institute in June 2019. RERF joined in the NEWS as a main collaborative institute, and Drs. Ohishi and Kitamura from the Department of Clinical Studies have cooperated in the clinical study as collaborative researchers. During 2019, we continued to recruit study participants to establish a cohort and, conduct general health examination in cooperation with JNIOSH. Thyroid ultrasound examination, questionnaire survey for psychological effects, structured interview to find out mental disorders, and screening tests of cataract were conducted continuously. We also continued re-evaluation of individual exposed doses.