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 F_1 (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, Molecular Biosciences, ITD, and Biosample Research Center. 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 31 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 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 F_1 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 health examinations of the FOCS every 4 years in November 2010 (the second round examination of the FOCS). We have completed the third round examination in September 2021. The fourth round examination that was initiated in November 2018 will be almost completed in FY2022.

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 lead research institute in June 2019. RERF has joined in the NEWS as a collaborative institute, and the Department of Clinical Studies has taken on the special clinical study. The Department of Clinical Studies has also cooperated in thyroid cancer study, cataract study, and psychological effects study of the NEWS. 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.

FY2021 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 completed gastric cancer study and are continuing work on liver cancer study in collaboration with not only the Statistics and Epidemiology Departments but also outside investigators.

[AHS liver cancer studies (Ohishi W)]

- A nested case-control study using stored sera showed that HBV and HCV infection and radiation exposure are associated independently with increased risk of hepatocellular carcinoma (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 such as CRP, TNF-α, IL-6, adiponectin, leptin, resistin, type 4 collagen, and platelet count. 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, 2020; 62).

  A preliminary analysis has revealed evidence of possible mediation of radiation risk for HCC by HBV. We have examined the suitability of analytical methods and validity of model assumptions for the mediation model. Because of the presence of missing data, principled methods for dealing with missing need to be employed. We have therefore also investigated how best to conduct multiple imputation for this study given the nested case- control design. In particular, we are studying how best to account for the design parameters in the missing-data imputation model in collaboration with Department of Statistics.

Previous studies demonstrated that the prevalence of hepatitis B surface antigen increased with radiation dose among the AHS participants, but no relationship was found between radiation dose and the prevalence of anti-HCV. We have examined HCC incidence during follow-up after HBV/HCV screening from 1993-1995 among AHS participants. The analysis of radiation effects on HCC incidence accounting for possible mediation by HBV and HCV was conducted in collaboration with the Department of Statistics. The proportion of total HCC risk for radiation mediated by HBV and HCV on the log hazard scale were 0.11 (11%) and 0.33 (33%), respectively. A manuscript describing viral mediation of radiation risk for HCC is in preparation.

Radiation and hematological malignancies

Two studies are being conducted using 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 insight regarding the mechanisms of radiation- induced myeloid malignancies. We are working on the hematological malignancy studies in collaboration with not only the Statistics and Epidemiology Departments but also outside investigators.

[Pathogenesis of Myelodysplastic Syndrome (MDS) (Miyazaki Y and Imaizumi M)]

 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 are conducting 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.

Whole exome sequencing of blood samples serially collected before and after MDS diagnosis in 17 subjects were successfully conducted with average depth of 200-fold. MDS clones were detected 4-22 years before diagnosis and expanded during the development of MDS. There are two patterns in clonal evolution; 1) sustained expansion of MDS clones which had chronal-hematopoiesis related alterations (ex. DNMT3A, TET2) and 2) remarkable clonal shift and/or rapid expansion of MDS clones which had complex karyotypes and del11q including ATM. The former pattern was mostly observed in the less- exposed survivors (<1Gy) and the latter one was mostly observed in the high-exposed survivors (>=1Gy).

[Chronic myeloid leukemia (CML) study (Yoshida N)]

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. We have conducted a preliminary study to assess whether formalin-fixed paraffin-embedded (FFPE) samples of 3 CML autopsy cases are available for molecular analysis. We performed

pathological analysis and extracted DNA and RNA from the unstained slides. Using the extracted DNA and RNA, we evaluated the existence of BCR-ABL1 and several genomic mutations with a diagnostic value for hematological malignancies.

PCR /RT-PCR/droplet digital PCR (ddPCR) and pathological analyses indicated that extracted DNA and RNA from FFPE samples are suitable for traditional molecular analysis. Analysis of ddPCR identified BCR-ABL1 in 2 of the analyzed cases, but not in the other case, suggesting that CML diagnosed using pathological criteria alone may result in misdiagnosis. A full-scale study for molecular and pathological analysis on early developing leukemia cases is planned. Additionally, we will conduct a feasibility study in which we will test whether DNA/RNA extracted from FFPE samples among early developing leukemia cases are applicable for targeted high-throughput sequencing.

[Radiation and Noncancer Condition: Cataract study (Hida A)]

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 conducted a new ophthalmological study to obtain lens images by 3 devices; slit- lamp, retro-illumination camera, and Scheimpflug camera with standardized method. Posterior sub-capsular and cortical opacities were scored with images obtained by retro-illumination camera, and nuclear opacities were evaluated quantitatively with images obtained by Scheimpflug camera.

- 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 1048 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. Scoring of cataract severity using photographed images was completed by an ophthalmologist and a statistical analysis was almost completed in collaboration with the Statistics Department. The presence or absence of cataracts was analyzed by inverse probability weighting logistic regression model (IPWLR) to account for possible missing data due to cataract surgery. The results of analysis suggested that cataract prevalence was significantly associated with age, sex, city, smoking, ultraviolet, and axial length. The significant association between radiation and posterior subcapsular cataracts was also shown. However, radiation effects on cortical cataracts and nuclear cataracts were not observed.

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 have conducted studies the objectives of which are to examine in detail the association between radiation exposure and atherosclerosis, heart disease, chronic kidney disease (CKD), stroke, and myocardial infarction.

[Atherosclerosis study, Part 1 (Nakamizo T)]

 Past studies have reported an association between radiation exposure and cardiovascular disease mortality/morbidity. Although the mechanism is unclear, a plausible one is radiation-induced atherosclerosis. To examine the association between radiation and atherosclerosis, we measured a comprehensive set of indicators of atherosclerosis including ankle-brachial index, carotid intima-media thickness, augmentation index, central systolic blood pressure, brachial-ankle pulse wave velocity, upstroke time, and aortic calcification evaluated from chest and lumbar X-rays among 3,775 AHS participants in 2010-2014. Data were analyzed by structural equation modeling with latent variables representing main atherosclerotic pathologies: 1) arterial stiffness, 2) aortic calcification, and 3) plaque.
Aortic calcification and plaque were linearly associated with radiation, but arterial stiffness was not related to radiation. The association was not so strong—comparable to about 2 years of aging per Gray of radiation exposure. The results of this cross-sectional study suggest a possible causative role of radiation on atherosclerosis, which should be confirmed by future longitudinal studies. A paper on the results has been published (Eur J Epidemiol, 2021; 36).

[Atherosclerosis study, Part 2 (Nakamizo T)]

 A recent study suggested a relationship with subclinical atherosclerosis. The mechanism is, however, unclear. In addition to potential involvement of inflammation to be investigated in the Clonal Hematopoiesis Program Project, we will investigate disturbance in vascular repair driven by the proliferation and differentiation of vascular (mesenchymal) stem/progenitor cells. This is a cross-sectional study among about 2,000 AHS subjects in Hiroshima. We have measured several multi-functional cytokines involved in the injury-repair system such as osteopontin, osteoprotegerin, and vascular endothelial growth factor (VEGF)-A. We are assessing the availability of the measured values, in terms of intra- and inter- reproducibility (coefficient of variation: CV), measurement error, and temporal intra-individual variation (intraclass correlation coefficient: ICC).

[Program project: Clonal hematopoiesis and inflammatory phenotypes potentially related to atherosclerosis risk in atomic-bomb survivors, project 2 (Nakamizo T)]

 Previous studies in A-bomb survivors suggest a relationship between radiation exposure and atherosclerotic diseases and inflammation. Recent evidence in studies in not involving radiation exposure suggests that clonal hematopoiesis (CH) can cause chronic inflammation leading to atherosclerotic diseases. To evaluate the hypothesis that CH caused by irradiation to hematopoietic stem cells is a cause of chronic inflammation and subsequent atherosclerosis in A-bomb survivors, we are initiating a study that analyzes stored AHS datasets relating hematological profiles with inflammatory and atherosclerotic indicators. The relevant data has been extracted from the RERF database.

[Heart disease study using echocardiography (Yoshimuta T, Hida A, and Nakamizo T)]

 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 subjects who were 15 years of age or younger at the time of bombing. We have obtained early indicators of heart disease, using echocardiography and relevant biomarkers to determine heart disease risks among 2,889 AHS participants. Reassessment of echocardiographic images are ongoing by a cardiologist of Nagasaki University.

[Chronic kidney disease (CKD) study (Sera N and Hida A)]

 Recent results from RERF showed that CKD diagnosed using only estimated glomerular

filtration rate (eGFR) is significantly associated with radiation dose among Nagasaki AHS participants. The analysis of the association between CKD diagnosed using urinary albumin and eGFR or albuminuria and radiation dose was continued for AHS participants in Hiroshima and Nagasaki.

Preliminary analyses showed that radiation dose was significantly associated with CKD and macro-albuminuria among AHS subjects in Hiroshima and Nagasaki after adjustment for age, sex, city, smoking and alcohol habit. However, these significant associations were lost after additional adjustments for CKD risk factors, which suggest possible mediating effects through CKD risk factors. The detailed statistical analyses are ongoing in collaboration with Department of Statistics.

[Stroke incidence study (Nakamizo T)]

 There is conflicting evidence whether sub-therapeutic irradiation is associated with stroke incidence/mortality. RERF has investigated the association in three studies, out of which two studies suggested radiation effects on stroke that differ among subtypes (Shimizu et al. BMJ 2010 and Takahashi et al. BMJ Open 2012). In those studies, however, some uncertainty existed regarding diagnostic accuracy and subtype classification. Accordingly, it remains unclear whether radiation exposure increases the incidence of stroke and/or its specific subtypes among A-bomb survivors. To evaluate whether exposure to radiation increases the incidence of fatal and nonfatal stroke and/or its subtypes among A-bomb survivors, we will conduct a retrospective cohort study on approximately 12,000 AHS participants in Hiroshima and Nagasaki. The outcome is incident non-fatal and fatal stroke and its major subtypes, CI, ICH, and SAH. Eligible participants will be followed from the inception of the cohort (1958) until 2013.

The RP has been approved by the Non-Cancer Research Cluster, outside reviewers, and internal committees. We have extracted the relevant data from the RERF database and started the chart review to identify stroke cases using standardized criteria.

Radiation and Noncancer Condition: Others

We are conducting additional studies focusing on the possible associations between radiation exposure and other noncancer diseases or condition at low-to-moderate doses as well. The LSS data have suggested radiation-related increased 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. Current studies exploring the relationship between radiation exposure and the development of the diseases are underway.

[AHS thyroid study (Imaizumi M)]

 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 are currently conducting 2nd cycle of thyroid examination such as blood tests and ultrasonography in younger exposed AHS participants including those exposed in utero. Analyses of 2nd cycle will be started in 2023 and results of incidence data are expected after 3rd or 4th cycles. We are also preparing the renewal of the RP including a method of longitudinal data analyses.

[Glucose and lipid metabolism study (Tatsukawa Y)]

 ABCC-RERF has conducted several dose-response studies regarding the association between radiation and diabetes among AHS participants, but the findings were inconsistent. We investigated the association of radiation dose with incidence of diabetes and evaluated whether the dose response is modified by other factors including city and age at time of bombing (ATB). The results showed that radiation dose was associated with diabetes incidence, however the association was modified by age ATB and city. A paper on the results has been accepted to an international journal (J Clin Endocrinol Metab, in press).

[Liver stiffness study (Ohishi W and Tatsukawa Y)]

 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. Discussions to examine associations between liver stiffness, radiation dose, and liver diseases have been started.

[Cognition among survivors exposed in childhood and in utero (Yamada M)]

 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) for objective cognition and the Neurocognitive Questionnaire (NCQ) for subjective cognition. Regarding NCQ, latent factors related subjective neurocognitive complaints were identified by an exploratory factor analysis among non-exposed subjects.

  The results indicated that there is no significant difference of objective and subjective cognitions between atomic bomb survivors and controls. A paper about radiation effects on objective cognition (CASI) among survivors exposed in utero (Am J Med, 2021; 134) and a paper about radiation effects on subjective cognition (NCQ) among survivors exposed in childhood (Radiat Res, in press) have been published. Selection bias related to study subjects is a limitation of the study, because subjects were consisted of health examination participants only and the participation rate was not high enough. Radiation effects on subjective cognition (NCQ) among survivors exposed in utero has been analyzed separately and a paper about the results was submitted for journal publication.

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:

[F1 offspring clinical study (FOCS) (Ohishi W, Tatsukawa Y, and Hida A)]

- The third-round examination of the F1 offspring clinical study (FOCS) that was initiated in November 2014 on a four-year cycle completed, 9,860 subjects participated by the end of September 2021 (participation rate of 75.3%). The fourth round was started in November 2018, 5,809 persons participated in the health examination by the end of November 2021 (participation rate of 58.4 %).
- We continued efforts to develop an integrated project in collaboration with Departments of Epidemiology, Molecular Biosciences, and Statistics. An umbrella project was organized for investigation of genetic effects of atomic bomb radiation. We continued to be involved in the umbrella project through the role of obtaining clinical and epidemiological information or biosamples from FOCS participants in preparation for future linking genome information with clinical epidemiological information.
- We have developed the analysis plan with Statistics Department's FOCS Analysis Working Group through monthly meeting to examine associations between parental radiation dose and the development of multifactorial diseases in the F1 offspring of A-bomb survivors.

Nuclear Emergency Workers Study (NEWS) [Okubo T and Ohishi W] 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. 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 lead research institute in June 2019. The research team consists of JNIOSH, RERF, and 11 collaborating research institutes, which conduct respective studies related to their specialties. To conduct research effectively, an operating committee and subcommittees, which may include working groups, will be established as needed. The subcommittees involve 8 major components: clinical study (Drs. Ohishi and Kitamura as collaborative investigators; Drs. Yamada and Imaizumi as cooperative investigators), cataract study (Dr. Hida as a cooperative investigator), thyroid cancer study (Dr. Imaizumi), psychological effects study (Dr. Yamada), cause-of death/cancer incidence study (Dr. Ozasa as a collaborative investigator), dose evaluation (Dr. Ozasa), health management database and analysis and evaluation. RERF joined in the NEWS as a collaborative institute, and we took on the clinical study as members of clinical study subcommittee.

The clinical study subcommittee (I) has examined creating the algorithm to automatically judge chronic hepatitis B or C and hypertension based on questionnaire (medical history and drug information), blood test and actual measurement as a preliminary study. We also supported implementation of the health study in collaboration with JNIOSH. A paper entitled "Baseline Survey of the Epidemiological Study of Health Effects in Fukushima Emergency Workers from 2016 to 2019" has been submitted to an international journal.