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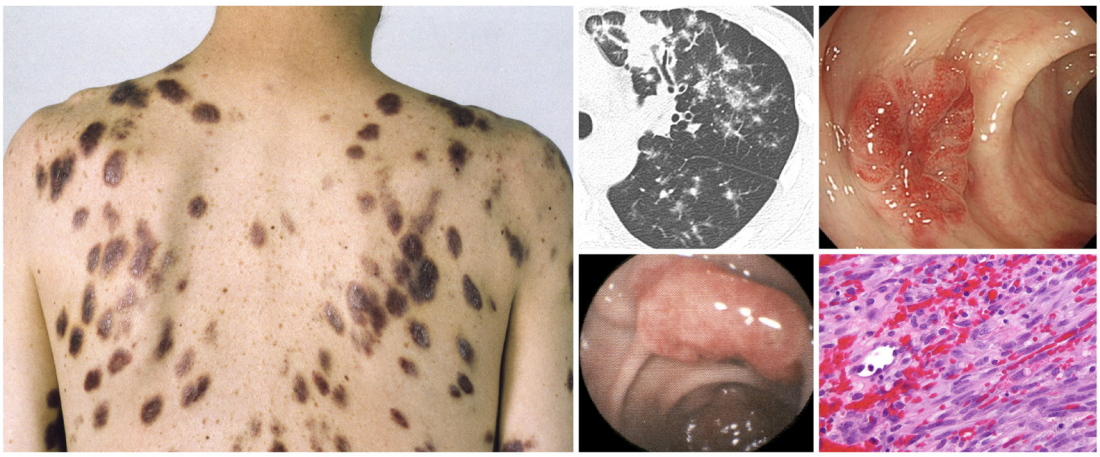

HIV-Associated Malignancies at 40: Much Accomplished but Much to Do

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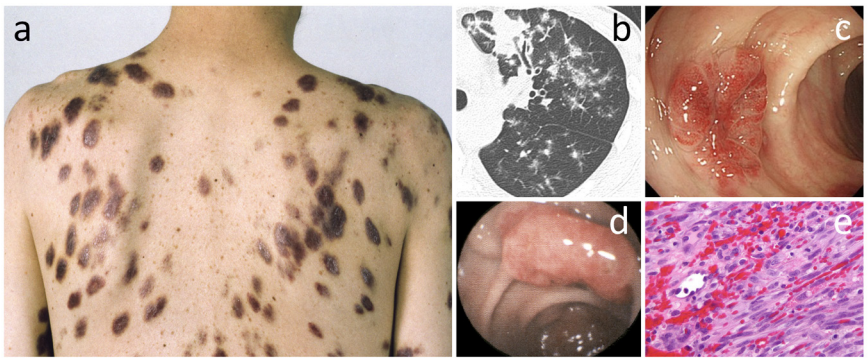

a. Multiple Kaposi sarcoma lesions appearing in the back of a patient with AIDS; **b.** Multiple Kaposi sarcoma lesions appearing in the lung; **c.** Kaposi sarcoma lesions appearing in the colon; **d.** Kaposi sarcoma lesions appearing in the stomach; **e.** Histology of Kaposi sarcoma showing the characteristic spindle cells, high vascularity, and intracellular hyaline globs (hematoxylin-eosin staining).

(Figures **a** and **d** are through the courtesy of Dr. *Bijan Safai*, while Figures **b**, **c**, and **e** of Dr. *Shin-ichi Oka*)

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EDITORIAL

HIV-associated malignancies at 40: much accomplished but much to do

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Abstract: The report in 1981 of a cluster of cases of Kaposi sarcoma (KS) in homosexual men in New York and California was one of the earliest harbingers of the AIDS pandemic, and association of cancer with HIV/AIDS has been one of the key features of this disease since. Looking back at year 40, the development of anti-retroviral therapy markedly reduced the incidence of AIDS-related cancers that occur at low CD4 counts, and this has been one of the most impressive advances in cancer prevention over the past half-century. There have also been advances in prevention and treatment of various HIV-associated tumors. However, as AIDS patients are living longer, there has been an increase in other cancers. Cancer continues to be one of the most frequent causes of death in persons living with HIV, and further basic, translational, clinical, and epidemiologic research in this area is urgently needed.

***Keywords*:** HIV, AIDS, Kaposi sarcoma, KSHV, PLWH

In July, 1981 the United States Centers for Disease Control reported a cluster of cases of Kaposi sarcoma (KS) in 26 homosexual men in New York and California (*1*). This report, along with another from the previous month describing 6 cases of community-acquired *Pneumocystis carinii* pneumonia were the first indications of the worldwide AIDS pandemic, which has since killed more than 36 million people worldwide. It soon became evident that this new immunodeficiency disorder, which was by 1984 shown to be caused by a novel retrovirus, human immunodeficiency virus (HIV), was associated with a marked increase in certain tumors, especially KS and high-grade B cell lymphomas, but not others. KS had previously been an extremely rare skin tumor in the Unites States, and its particular association with HIV/AIDS in gay men was initially quite puzzling. However, in 1994, the team of Patrick Moore and Yuan Chang showed that KS was caused by a novel gammaherpesvirus, which they named Kaposi sarcoma-associated herpesvirus (KSHV) and is also referred to as human herpesvirus-8 (HHV-8) (*2*). With this discovery, it became clear that most of the tumors whose incidence is increased in HIV/AIDS are caused by oncogenic tumor viruses, especially KSHV, Epstein Barr virus (EBV), and human papillomavirus (HPV) (*3*). We now know that KSHV is excreted in saliva and that the marked association between KS and AIDS was because of an otherwise silent epidemic of KSHV in gay men that was occurring at the same time as the HIV pandemic.

For some time after its recognition as a new disease, AIDS was almost always a death sentence; patients usually died within a couple of years, and a high percentage of these deaths were from AIDS-associated cancers. The first breakthrough came with the development of the initial antiretroviral drugs: AZT (zidovudine) and other nucleoside reverse transcriptase inhibitors (*4,5*). These increased the CD4 counts and significantly increased the survival of patients with AIDS, especially when used in combination. In addition, they reduced the incidence of HIV-associated malignancies. However, resistance to these early antiretrovirals often developed. The subsequent development and widespread use of HIV protease inhibitors starting around 1996 enabled the advent of 3-drug regimens which could essentially completely thwart HIV replication and changed AIDS into a chronic manageable disease. In addition, combination antiretroviral therapy (ART) markedly reduced the incidence of AIDS-related tumors that occur at low CD4 counts (Table 1), and in fact this has been one of the most impressive recent advances in cancer prevention in the past 50 years (*6*). Along with this, we have seen marked advances in the treatment of HIV-associated cancers. These advances have in part been enabled by the development of new anti-cancer drugs and regimens, and in part by the immune restoration caused by ART, which has enabled the use of full-dose therapies used in the general population. In particular, there have been dramatic improvements in the survival of PLWH with high-grade B cell lymphomas, with KS, and with a form of multicentric Castleman disease caused by KSHV (*3*).

At the same time, along with their increased longevity, PLWH are experiencing more cancers that

Table 1. Estimated numbers of selected cancers in people living with AIDS in the United States in different time periods

Association	Cancer	1991-1995	1996-2000	2001-2005
Associated with low-CD4 counts	Kaposi sarcoma	21,483	5,727	3,827
	Non-Hodgkin lymphoma	12,778	7,292	5,968
Some immunologic association	Cervical carcinoma	327	419	530
	Anal cancer	206	770	1,564
	Hodgkin lymphoma	426	682	897
	Lung cancer	875	1,383	1,882
No immunologic association	Colon cancer	108	230	438

Estimated numbers of cancers in people living with AIDS in the United States in different time periods: pre-three drug antiretroviral therapy (1991-1995); early post three-drug therapy (1996-2000); and later post three-drug therapy (2001-2005). From Shiels *et al*., (*6*).

are associated with advancing age (*6*). These include increases in certain other HIV-associated tumors that are not strongly linked to low CD4 counts, such as anal cancer or lung cancer (Table 1). In addition, along with the increasing number of older patients with HIV infection, there are increases in other common cancers that are not linked to immune defects in this population, such as prostate cancer or breast cancer. And, we continue to see severe cases of KS and other AIDS-related tumors associated with significant immunosuppression among patients who are not diagnosed with HIV until late in the course of infection. Cancer is now one of the most frequent causes of death in people living with HIV (PLWH) in developed nations. Moreover, HIV-associated malignancies are a major health problem in sub-Saharan Africa and other resource-limited regions; in some countries in sub-Saharan Africa, KS is the most common tumor overall in men (*7*). And in resource rich countries, like the United States, KS and other HIV-associated malignancies disproportionately affect individuals experiencing health disparities (*8*).

So while much progress has been made, there is much to do.

The most important task of course is to end the HIV/AIDS epidemic. Development of an effective HIV vaccine would help greatly, but this has so far been an elusive goal. Meanwhile, implementation of strategies including safer sex education, testing in high-risk settings and pre-exposure prophylaxis for those at risk can substantially reduce HIV transmission. Also, ensuring prompt initiation of HIV therapy and continuity of HIV care to support adherence will result in viral suppression, leading to the reduced sexual transmission of HIV. With regard to HIV-associated and other malignancies in PLWH, prevention is also essential. As noted, treatment of HIV with ART can dramatically reduce the incidence of tumors associated with profound immunosuppression. Appropriate screening for other cancers associated with advancing age and exposure to oncogenic viruses will be pivotal. Cervical cancer, caused by HPV and preceded by precancerous lesions, can be prevented by screening but this can be challenging in resource-poor regions; it will be important to optimize screening strategies in these regions and implement their use. While anal cancer is also preceded by precancerous lesions, it is unclear if treating these lesions is an effective strategy; the ANCHOR (Anal Cancer HSIL Outcomes Research Study) being conducted in the U.S. AIDS Malignancy Consortium is addressing this question. Lung cancer is now the most common cause of cancer-related death in PLWH receiving ART in the United States (*9*), and cigarette smoking prevention and cessation are among the most important prevention strategies. Studying cancer screening and prevention strategies in PLWH in other cancers increasing in incidence, such as hepatocellular carcinoma is essential. Vaccination strategies, particularly for HPV and hepatitis B, have also been shown to markedly reduce cancers caused by these viruses. The HPV vaccine has been shown to substantially reduce the incidence of cervical cancer when widely administered before sexual debut (*10*). And if effective vaccines against EBV and KSHV could be developed, we could eradicate the cancers caused by these gammaherpesviruses.

There is still much we do not know about the pathogenesis of HIV-associated cancers, and it is essential that we continue to pursue knowledge in this area through basic and translational research. This research can then inform advances in therapy of HIV-associated and other tumors developing in PLWH. While we have made great strides in the treatment of certain HIV-associated tumors, others such as primary effusion lymphoma, advanced anal carcinoma, and lung cancer, still carry a relatively poor prognosis in this population, and improved therapies are urgently needed. One promising approach is the development of specific therapies targeted at cellular mutations and/or virally-encoded genes that drive cancer development. Also, one of the most exciting advances in cancer treatment in recent years is checkpoint inhibitors and other immune therapies. We now know that checkpoint therapy is safe and can be effective in PLWH (*11*), and it will be important to continue to investigate this modality and other approaches to harness the patient's immune

system to fight HIV-associated malignancies. In this regard, PLWH are increasingly developing common tumors such as colon, breast, and prostate cancer, and it will be important to make cancer clinical trials open to PLWH whenever possible. And since the vast majority of PLWH now live in resource-limited regions, it will be important to develop treatment strategies that can be implemented in those regions.

Reflecting back after 40 years, it is remarkable how much progress has been made in HIV-associated malignancies, in part stemming from progress in HIV care and in part from progress in cancers themselves. At the same time, cancer continues to be one of the most common causes of death in PLWH, and there is much work to do.

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REVIEW
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International strategy in cancer epidemiology: Japan's involvement in global projects and future role

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Abstract: In recent years, collaboration among researchers in the field of cancer epidemiology has been accelerating in various forms. Here, we review recent trends in international collaborative research activities in the cancer epidemiology field in Japan. These include not only support for other countries with less developed cancer statistics infrastructures, but also large-scale compilations and international comparisons through collaborative studies, as well as integration with analytical epidemiology and clinical research. Formation of international cohort consortia and estimates of cancer and risk factors in each country have contributed to raising the skill levels of cancer epidemiologists as well as to expanding research networks and activities among cancer epidemiologists. Molecular and genome epidemiological studies on cancer have progressed over decades and these continue to increase in size and dimension. Application of evidence from this area in prevention is still underway and needs further effort. Japanese epidemiologists have great potential to assume international leadership roles by taking advantage of the uniqueness, originality and characteristics of Japanese cohorts.

***Keywords*:** cancer, epidemiology, international collaboration

Introduction

Because of the major regional and global disease burden of cancer, cancer epidemiology has grown in importance and now covers a wide range of research aimed at both identifying the causes of cancer and preventing cancer at the population level. To achieve these, it is essential to understand the geographical distribution of cancer and its time trends, and to clarify the risk factors for prevention. Specifically, population-based cancer registration is an inevitable basis for descriptive statistics of cancer while analytical epidemiological research is crucial to elucidating the causes of cancer. Genomic epidemiological methods have attracted attention in recent years. These different research approaches will be bridged with health policy and practice using individual epidemiological evidence, systematic review and meta-analysis, pooled analysis, and evidence-based cancer prevention methods, as well as research into the dissemination and implementation of their findings.

Epidemiological research on cancer is no longer irrelevant to globalization, and the identification and control of risk factors is a common issue both domestically and overseas. In recent years, collaboration among researchers in the field of cancer epidemiology has accelerated in various forms. Achieving cancer prevention now requires the creation of high-impact evidence, and large-scale research collaboration platforms have emerged for that purpose. Japan is no exception; the quality of epidemiological research and researchers has improved dramatically in the last few decades. Considered in terms of the evidence-building required to realize effective cancer control policy, the cancer epidemiology field in Japan has, in a sense, entered a mature stage.

Here, we introduce recent trends in international collaborative research activities in the cancer epidemiology field in Japan, including current achievements and future prospects.

International collaborative activities in the field of descriptive epidemiology in cancer

The development of cancer registries and mortality statistics is proceeding at a rapid pace all over the world. The first population-based cancer registry (PBCR) was launched in Germany in 1929, followed by the US and Denmark (Table 1). In Japan, the first PBCR was organized by Miyagi prefecture in 1955, followed by Hiroshima city, Nagasaki city, and Aichi, Osaka

Table 1. History of population-based cancer registry and related events

Year	Population-based cancer registry in the world	Population-based cancer registry and related events in Japan
1899		The Vital Statistics Survey began to be conducted centrally using individual votes
1929	Germany (Hamburg)	
1940	USA (New York State)	
1941	USA (Connecticut)	
1942	Denmark	
1944	Canada (Saskatchewan)	
1945	England and Wales (SW)	
1948	England and Wales (Liverpool)	
1948	New Zealand	
1950	Canada (Manitoba)	
1929	Slovenia	
1950	Canada (Alberta)	
1951	USA (El Paso)	
1951		PBCR in Miyagi (the first regional PBCR)
1957		PBCR in Hiroshima city
1958		PBCR in Nagasaki city
1968		PBCR in Aichi and Osaka
1970		PBCR in Kanagawa
2006		Enforcement of the Cancer Control Act
2013		PBCR in Miyazaki (the 47th regional PBCR out of the 47 prefectures)
2016		Enforcement of the Act on Promotion of Cancer Registries

and Kanagawa prefectures. PBCRs covered the entire population in 2013. The development of mortality statistics goes back a long way, and the Vital Statistics in Japan was started in 1899. The role of descriptive epidemiology is to plan cancer control measures based on an understanding of the actual status of cancer burden. In many low- and middle-income countries (LMICs), cancer statistics do not exist, and evidence-based cancer control measures cannot be implemented, even though the burden of cancer is increasing. The global initiative for cancer registry development, launched in 2011 and led by the International Agency for Research on Cancer (IARC), has established hub centers in five continents. The fruit of almost 10 years' activities includes 167 site visits, 17 agreements and 89 training courses to date. The initiative has been deemed a success. Japan is involved in this project as a collaborating center in Asia, supporting the hub center at the Tata Memorial Cancer Center, Mumbai, India, and working to develop cancer statistics in Southeast Asian countries. Vital Strategies, a U.S. consulting firm, is developing a project called Civil Registration and Vital Statistics for Asian countries, which provides support for the collection of mortality information (*https://www.vitalstrategies.org/programs/civil-registration-and-vital-statistics*). In parallel, the World Health Organization (WHO) is implementing the Global Initiative for Childhood Cancer project (*1*), with the aim of reaching a survival rate for children with cancer of at least 60% by 2030, and statistics on childhood cancer are becoming more accurate. In this manner, through collaboration among international organizations, the private sector, and academia, certain results have been achieved. In Southeast Asian countries such as Vietnam and Myanmar, high-level cancer statistics have been developed in the space of only a few years. In previous years it typically took at least 10 years from the time a cancer registry was launched to achieving stable operations, but this has been shortened to 3 to 4 years. In addition, even in a country with a huge population like China, more than 600 cancer registries have been established to provide accurate cancer statistics for use in active cancer control (*2*).

Through these support efforts, cancer statistics have been developed in many countries, and accurate cancer statistics can be compared across more regions (*3*). Epidemiological data are available from WHO and IARC for incidence (Cancer Incidence in Five Continents), mortality (Mortality Database), and survival (SURVMARK and SURVCAN). Estimates have also been calculated for regions where actual data are not available in the GLOBOCAN project (*4*). GLOBOCAN 2020 estimates that there were 19,292,789 new cases of cancer worldwide, 9,958,133 deaths from cancer, and 50,550,287 5-year prevalent cases in 2020. Lung is the most common cancer in men (14.3%) and breast in women (24.5%). In addition to international organizations, acadamia have also started large-scale international descriptive epidemiological studies. With regard to survival, the University of London is leading the CONCORD Study (*5*), and the RARECAREnet study is focused on rare cancers, mainly under the direction of the National Cancer Institute in Milan, Italy. For rare cancers in Asia, the RARECAREnet Asia study has been initiated, led by the National Cancer Center Japan, with the participation from Japan, Korea, Taiwan, Thailand, Malaysia and India (*6*). The proportion of rare cancers in overall incidence was 16.3% in Japan, 23.7% in Korea, 24.2% in Taiwan and 22.2% in the EU. Numbers of newly diagnosed rare cancer cases in 2015 were 140,188 in Japan, 52,071 in Korea, and 24,147 in Taiwan. A

new barrier to international research is the exchange of medical information; in a sense, overreaction to the promulgation of the General Data Protection Regulation (GDPR) has made it difficult to aggregate and exchange individual cancer data in many countries. For this reason, attempts have been made in the last couple of years to create statistical models which aggregate data without taking individual data out of the country that produced it (*7*). This method, called Distributed Learning or Federated Learning, outputs only the coefficients of the equation, and no personally identifiable information leaves the local computer or storage area outside the facility.

In many developed countries, descriptive epidemiology on cancer is not limited to the calculation of cancer statistics and their use in cancer control. Rather, it is also being integrated with analytical epidemiology and clinical research fields, and has begun to influence the identification of cancer risk factors and determination of medical treatment policies. These attempts aim to sublimate the strengths of descriptive epidemiological information, such as population-based cancer registries and mortality statistics, into analytical epidemiology through linkage between databases, while taking advantage of their completeness, risk population identification, unbiasedness, and standardized collection items. Interdisciplinary integration is often carried out by merging socioeconomic databases, such as census data for risk factors of cancer incidence and mortality, with cancer registries and mortality statistics for outcomes, or by adding detailed medical information to cancer registries and mortality statistics. In the Netherlands and Scandinavian countries, multiple cancer-related databases have already been linked in real time for several decades, and the boundary between descriptive and analytical epidemiology has effectively vanished. In Norway and Denmark, studies with these designs which linked census data and data from cancer registries to analyze the relationship between occupational exposure to carcinogens and cancer incidence were conducted as early as the 1980s (*8*). Moreover, a number of studies in the Netherlands have added clinical information to cancer registry data (*9*).

Descriptive epidemiology on cancer is now a standardized and accurate way to determine the cancer burden worldwide, and international collaborative research has been conducted through the cooperation of many countries. The development of technology to promote research while ensuring the protection of personal information is also remarkable. On a more granular level, analytical epidemiological approaches which integrate cancer statistics with other statistics have been taken. Behind all these trends is the rapid spread of computers and the development of high-speed networks that can be used by everyone. In Japan, integrating databases that were developed independently is extremely difficult, and these stand like an urban landscape of inconsistent buildings. LMICs should take advantage of their latecomer status by learning from these negative lessons and moving forward with a firm focus on the development of integrated cancer statistics.

To summarize, various aspects of international research collaboration have been activated over a number of decades in the field of descriptive epidemiology of cancer. These have aimed to support countries, especially LMICs, which have yet to develop a robust cancer statistics infrastructure; conduct large-scale compilation and international comparisons through collaborative studies; and integrate with analytical epidemiology and clinical research.

Research collaboration platform: cohort consortia and risk factor burden analysis

Evidence from epidemiological studies on the association between lifestyle and cancer risk has increased in the last few decades, and understanding of cancer etiology continues to grow. Notable recent trends in large-scale cohort studies are the formation of cohort consortia and the active progress of pooled analyses. To make efficient use of existing cohort studies around the world and to achieve more precise estimates, cohort consortia with major risk factors and major outcomes have been established, mainly under the leadership of the US and Europe. The Pooling Project of Prospective Studies of Diet and Cancer (DCPP) ($n \approx 800{,}000$) (*10*) centered around Harvard University has pooled cohorts mostly from the US and Europe, as well as some from Asia, including Japan, to analyze the association between dietary factors and cancer (*11*). The European Prospective Investigation into Cancer and Nutrition (EPIC) study (*12*) ($n \approx 500{,}000$) is a large-scale cohort consortium across European countries which is unique for its multiple cohorts which were each established using a common protocol from the planning stage.

In Asia, the Asia Cohort Consortium (ACC) (*13*), established in 2004 by cancer epidemiologists across the Asian region, has expanded its contribution to collaborative research across the Asian region in the cancer epidemiological research field (*14*) (Figure 1). It aims to understand the association of genetic and environmental factors with the onset of disease using cohorts from Asian countries with a total healthy population of over one million, and to utilize this cohort data to provide reliable scientific evidence on emerging health issues and causes in Asia. The ACC has two missions: to serve as a platform for cross-collaborative projects and combined analysis in Asia, and to act as an incubator for new cohorts. The latter is unique to ACC in that it has the role of not only conducting pooled analyses, similar to many cohort consortia in Europe and the US, but also of providing intellectual support (methodology and common research materials) - albeit not funding - to the creation of new cohorts. Particularly

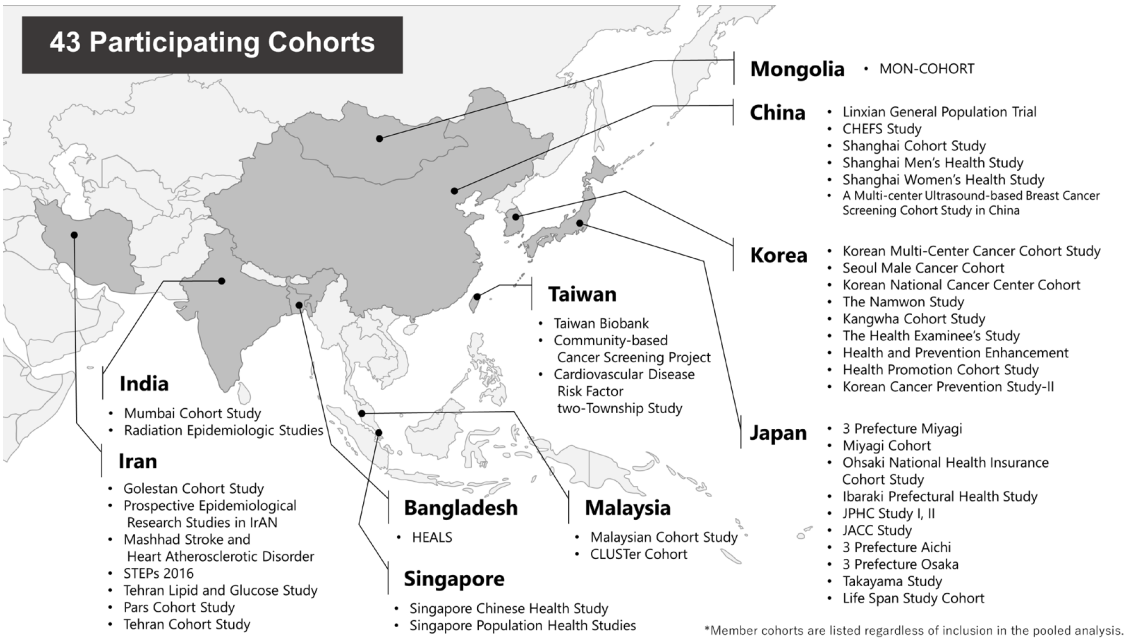

Figure 1. Participating cohorts of the Asia Cohort Consortium (as of April 2021) (*https://www.asiacohort.org/index.html*) (*13*).

in countries still developing their epidemiological resources, both human and non-human, this type of "regional" incubation is inevitable. Currently, 10 large-scale cohorts in Japan have joined to conduct pooled analyses, providing the largest population size among countries in the ACC. The ACC coordinating center is located in the National Cancer Center Japan and is in charge of the administrative work necessary to facilitate the various research activities conducted to fulfil the missions of the ACC.

The major strength of cohort consortia is large sample size. Larger sample sizes allow the investigation of uncommon or multiple types of exposure, rare diseases, and variation among population subgroups or races due to the greater statistical power provided over individual studies (*11*). Additionally, collection of individual data from each cohort allows reanalysis using standardized common confounding factors. This method supplements the limitations of meta-analyses from the published literature, which summarize risk estimates obtained for heterogeneous exposure categories with differing adjustment for potential confounders (*15*).

It is also noteworthy that studies into the attributable causes of cancer - estimated using population attributable fraction (PAF) in each country - are being widely promoted in many countries. Attributable causes for cancer differ between western countries and Japan. For example, PAF in the UK in men and women is 17.7% and 12.4% for smoking, followed by overweightness and obesity at 5.2% and 7.5% (*16*). In Japan, in contrast, tobacco smoking has the highest PAF (29.9%) followed by infectious agents (22.8%) in men, while infectious agents have the highest PAF (17.5%) followed by tobacco smoking (6.2%) in women (*17*). These discrepant findings highlight the importance of broad geographical confirmation of risk factors in multiple studies with large sample sizes.

The PAF estimates require the national representative prevalence of target risk factors; summary relative risk values from systematic reviews, meta-analyses, and/or pooled analyses; and nationwide cancer incidence and mortality statistics. Until now, PAF estimates on cancer have been reported from different countries and regions, including the US (*18*), Nordic region (*19,20*), UK (*16,21*), France (*22,23*), South Korea (*24*), China (*25*), Australia (*26*), Canada (*27*), Germany (*28*), and Brazil (*29*), as well as Japan (*17*), facilitated by the sharing of methodology. Research into the attributable causes of cancer through estimation of PAF provides evidence that has a direct impact on the health policy of the reporting country.

In addition, vigorous research into the global burden of disease is now underway for various diseases. The Global Burden of Diseases (GBD) (*30*) is the largest project; this estimates the global burden of disease from various perspectives by accumulating all available health-related data from each country into the Institute for Health Metrics and Evaluation (IHME), located at the University of Washington in Seattle. The burden of disease, globally and in each country, is estimated from various perspectives. With substantial research funding enabling independent operation, the GBD has become a huge consortium project with more than 3,600 experts from 160 countries around the world. The results from this project are published in The Lancet on a topic-by-topic basis and are influencing global health policymaking.

In summary, recent trends in the establishment of international cohort consortia and estimates of cancer and risk factors among countries have contributed to increasing the skill level of cancer epidemiologists,

as well as to expanding and strengthening research networks and activities among cancer epidemiologists.

Participation in cohort studies collaboration

Beginning in the 1980-90s, a number of large-scale population-based cohort studies with populations of more than 30,000 subjects in each cohort have been established in Japan. Currently, these cohort studies have follow-up times of 20-30 years, and most have reached the fruitful period in which they are able to yield epidemiological evidence of cancer. These cohort studies have also contributed to collaborative analyses in various research consortia platforms of cancer. To give one example, the Japan Public Health Center-based Prospective Study (JPHC Study), launched in 1990, consists of over 100,000 residents aged 40-69 years across Japan who have provided information on lifestyle habits and health conditions in multiple follow-up surveys. The study has participated in several international cohort consortia (*31*).

Although international cohort consortia have larger sample sizes, their simple aggregation into a single dataset is not feasible without the application of vast amounts of ingenuity. For example, two-stage analysis is opted for in the DCPP (*11*), one of the cohort consortia mentioned above. In the first step, these investigators calculate study-specific relative risks. In the second step, they conduct pooled analyses using a random- or mixed-effects model. An author in the DCPP mentioned the following in a methods paper (*11*): "Although combining the data from all studies is one way to take advantage of differences in the distributions of the exposure variable across studies, it assumes that the exposure was measured in comparable ways across studies. Because the distributions of dietary variables may differ across studies due to true differences in actual intake and due to differences in the dietary assessment methods used (and other study-specific sources of error), this assumption may not be reasonable, except for nutrients that come from a small number of food sources (*e.g.*, alcohol). In addition, combining the studies into one data set assumes that there is no between-studies heterogeneity in the associations of the outcome with the exposure or any of the covariates." This group has conducted both pooled and aggregated analyses and confirmed that the results were substantially comparable. Due to difficulties in testing the assumption, however, they generally conducted two-stage analysis. Although international cohort consortia have larger samples, it is necessary to consider differences in diet habits or assessment between countries or studies.

We recognize, however, a unique advantage in cohorts established in the Japanese population, because of differences between countries. Several examples may be cited. One example is in the presence of substantial differences in the traditional diet. The Endogenous Hormones, Nutritional Biomarkers and Prostate Cancer Collaborative Group (EHNBPCCG), which conducts collaborative analyses of individual participant data from prospective studies on the association of circulating hormone and nutritional biomarker levels with risk of prostate cancer. Several epidemiological studies reported that soy and isoflavone intake are possible preventive risk factors of prostate cancer (*32*). To examine the association between prediagnostic concentrations of circulating isoflavones and the risk of prostate cancer, two Japanese and five European prospective studies provided blood level data, including the JPHC Study. These showed large differences in circulating isoflavone concentrations between Japanese and European populations; for example, the mean genistein concentration in controls in Japanese studies (294.0-454.4 nmol/L) was more than 50 times higher than in European studies (5.19-5.61 nmol/L). It was therefore not possible to analyze these together; rather, the association of isoflavone concentration and prostate cancer was analyzed separately in Japanese and Europeans. Results showed no association in European men, but a high equol concentration was associated with a lower risk in Japanese men, with an OR in the highest quartile (95% confidence interval) compared with the lowest of 0.61 (0.39-0.97)). This international cohort consortium has suggested that further research is necessary in populations with high isoflavone intake (*33*). Further, this experience suggests that the presence of an association between a unique habit and several cancers can only be analyzed in the country or countries in which the habit is unique.

Another example is the existence of different incidence rates. The Biliary Tract Cancers Pooling Project consisted of 27 prospective cohorts with over 2.7 million adults, including the JPHC study. Biliary tract cancer incidence rates are subject to large worldwide variation. Rates are low in several European countries and the United States, but relatively high in Latin America and Asia, including Japan. Age-standardized incidence rates per 100,000 (world standard population) of gallbladder cancer and extrahepatic bile duct cancer - a subtype of biliary tract cancer - are 9.3 and 0.5 in Chile (men) and 2.4 and 3.7 in Japan (men) versus 0.3 and 0.4 in the UK (men) and 0.5 and 0.6 in the US (men), respectively (*34*). In fact, the Biliary Tract Cancers Pooling Project showed that the incidence rate of gall bladder cancer is much higher in the JPHC (10.4 per 100,000 person-time) than in European (EPIC, 2.0 per 100,000 person-time) and USA cohorts (NIH-AARP, 3.1 per 100,000 person-time). Japan's participation contributed to this project (*35*). Additionally, the association between body mass index and extrahepatic bile duct cancer was similar between this international cohort consortium (*35*) and a single report from the JPHC (*36*), with both showing that obesity may increase the risk of extrahepatic bile duct cancer. This suggests it is possible to provide sufficient evidence from one

country by taking advantage of the characteristics of each of the involved countries. Since infection is a major attributable cause of cancer in Japan, and the incidence of infection-related cancer, such as gastric cancer and liver cancer, is relatively high in Japan compared with Western countries, it may be advisable for Japan to take international leadership for these cancers.

In summary, although combining results from different cohorts is not a simple matter and should be done with caution, a larger sample size is one of the most important advantages of international cohort consortia. The resulting evidence - derived from populations around the world - is robust, and useful for ensuring the health of each of the populations involved. Experience with the JPHC Study indicates that Japanese cohorts have unique characteristics which differentiate them from other populations, namely in exposure distributions, such as dietary habits and prevalence of infections, and outcome features, including cancer types, and that investigators familiar with them may be candidates for leadership positions in international consortia.

Molecular and genome epidemiology research network

Molecular and genome epidemiological studies offer the possibility of investigating the impact of gene-environment interaction on ordinary environmental factors. These have recently been initiated as one area of oncology epidemiology with the aim of clarifying carcinogenic processes (*37*). These studies have helped clarify the significance in humans of findings from histopathological and experimental findings in carcinogenesis models in animals. Hypothesis-based research approaches, such as the association between functional ALDH2 polymorphisms and risk of drinking on esophageal cancer (*38*), have been favored. In terms of study design, the invariance of genetic factors has led to the reinstatement of the case-control study design, which was losing popularity in the evaluation of environmental exposures.

Advances in human genetic measurement techniques in the last 20 years, such as scanning gene polymorphisms array or next-generation sequencing and arrays, have significantly changed the approach of research in this area. It has lowered prices and enabled larger study sizes to be examined, and further resulted in the explosive enrichment of genetic information - examples include the Human Genome Project (*39*), International Hap-Map Project (*40*), and ENCODE projects (*41*). Based on these, research approaches have noticeably changed, from a hypothesis-based approach to a genome-wide, non-hypothesis-based one. For example, Genome-wide Association Studies (GWASs) of lung cancer made it possible to find genes such as telomerase, which cannot be found with a hypothesis-based approach (*42*). On the other hand, it was also interesting to discover genes that are likely to appear even in hypothesis-based studies, such as gene polymorphisms in the nicotine-like cholinergic receptor gene group on chromosome 15 (*43*). Most of the susceptibility loci identified from GWASs are on genes which might never have been identified through a conventional hypothesis-based approach, warranting the effectiveness of this approach, to a certain extent at least.

GWASs may be characterized as large-scale research employed to find gene polymorphisms with high prevalence but low effect size. The formation of a consortium centered on case-control studies of lung cancer, pancreatic cancer, breast cancer, head and neck cancer, ovarian cancer, pancreatic cancer, etc., which had been underway at that time played a major role in this. As one example, the University of Cambridge-led breast, ovarian, and prostate cancer consortium formed COGS (*44*). This consortium, based on a custom array called the COGS chip, was a hugely successful exemplar of so-called "big science", and led to the GAME-ON initiative (*45*). Each consortium in GAME-ON still aims to expand the extent of collaboration. More recently, the International HundredK+ Cohorts Consortium (IHCC) was established in 2018. This consortium aims to create a global network for translational research that utilizes large cohorts to enhance understanding of the biological and genetic basis of disease and improve clinical care and population health (Table 2) (*46*).

In this trend to increasing scale, attention has focused on the uniqueness of research into other populations, beyond Caucasians in Europe and the United States, such as those of Asian and African descent. For example, a GWAS meta-analysis of pancreatic cancer identified a GP2 gene polymorphism which is prevalent only in East Asians (*47*), and it has become clear that new ones can be found by changing the population. This shows the importance of creating a framework for collaboration among research groups from countries that have not previously formed such consortia.

Also noteworthy is the subdivision of diseases. Risk factors - especially genomic factors - that take account of the characteristics of tumors are being investigated, such as driver mutations in the EGFR gene for lung cancer (*48,49*) and the presence or absence of estrogen receptors in breast cancer (*50*). The need for larger-scale research to carry out these activities is increasing, and this trend will continue in the future.

In summary, molecular and genome epidemiological studies have progressed over the past few decades and continue to gain in size and dimension. Although outside the scope of this review, the application of evidence from this area to prevention is still underway, and further effort is required.

Conclusion

In this review, we introduced recent trends in

Table 2. Participating cohorts of the International HundredK+ Cohorts Consortium (IHCC) (*https://ihccglobal.org/membercohorts*) (*46*)

Cohort Name	Country/Region
23 and Me	USA
45 and Up Study	Australia
Africa Health Research Institute (AHRI) Population Cohort	South Africa
Apolipoprotein MORtality RISk study (AMORIS)	Sweden
Biobank Japan	Japan
BioVU Vanderbilt	USA
California Teachers Study (CTS)	USA
Canadian Partnership for Tomorrow's Health (CanPath)	Canada
Cancer Prevention Study II (CPS-II)	USA
Cancer Prevention Study II Nutrition Cohort	USA
Children's Hospital of Philadelphia (CHOP) Biorepository	USA, Europe, South America, Canada, Saudi Arabia, Australia
China Kadoorie Biobank	China
China PEACE (Patient-centered Evaluative Assessment of Cardiac Events) Million Persons Project	China
Constances Project	France
Danish National Birth Cohort	Denmark
East London Genes and Health	UK
ELSA-Brasil	Brazil
Environmental influences on Child Health Outcomes (ECHO) Cohort	USA
EPIC (European Prospective Investigation into Cancer, Chronic Diseases, Nutrition and Lifestyle)	UK, Italy, France, Germany, Norway, Nether-lands, Denmark, Spain, Greece, Sweden
EpiHealth	Sweden
Estonian Genome Project	Estonia
Finnish Maternity Cohort Serum Bank	Finland
Geisinger Cohort - MyCode Community Health Initiative	USA
Generations Study (GS)	UK, England, Scotland, Wales, Northern Ireland, Isle of Man, Channel Islands
Genomics England / 100,000 Genomes Project	England
German National Cohort (NAKO)	Germany
Golestan Cohort Study	Iran
Healthy Nevada	USA
Israel Genome Project	Israel
Japan Public Health Center-based Prospective Study (JPHC)	Japan
Japan Public Health Center-based Prospective Study for the Next Gen-eration (JPHC-NEXT)	Japan
Kaiser Permanente Research Program on Genes, Environment, and Health	USA, California
Korea Biobank Project	Republic of Korea
Korean Cancer Prevention Study (KCPS-II Biobank)	Korea
Korean Genome and Epidemiology Study (KoGES)	South Korea, Vietnam, Cambodia, Japan, China
LifeGene (and sister cohort, EpiHealth)	Sweden
LIFEPATH (Lifecourse biological pathways underlying social differences in healthy aging)	Europe, Australian, USA
Malaysian Cohort	Malaysia
Maule Cohort (MAUCO Study)	Chile
Mexico City Prospective Study	Mexico
Million Veteran Program	USA
Million Women Study	England, Scotland
Multiethnic Cohort Study (MEC, NCI)	USA, Hawaii, California
Netherlands Twin Registry	Netherlands
Newfoundland 100K Genome Project/Sequence Bio	Canada, Province of Newfoundland and Labrador
NHS (Nurses' Health Study, NCI)	USA
NHSII (Nurses' Health Study II, NCI)	USA
NICCC	Israel
Northern Sweden Health and Disease Study	Sweden
Norwegian Family Based Life Course Study	Norway
Norwegian Mother and Child Cohort Study (MoBa)	Norway
Pakistan Genomic Resource (PGR)	Pakistan
PERSIAN Cohort Study	Iran
PLCO (Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, NCI)	USA
Qatar Genome Project	Qatar
SAPRIN (South African Population Research Infrastructure Network)	South Africa
Saudi Human Genome Program	Saudi Arabia
Saudi National Biobank	Saudi Arabia
Shanghai Men and Women's Health Study (2 cohorts)	Shanghai, China
Singapore National Precision Medicine Program	Singapore
South(east) Asian Cohorts - NETWORK	Bangladesh, Malaysia, Sri Lanka
Taiwan Biobank	Taiwan
Trøndelag Health Study (HUNT)	Norway
U.S. Precision Medicine Initiative/All of Us	USA
UK Biobank	England, Scotland, Wales
UK Blood Donor Cohorts	UK
UKLWC (UK Collaborative Trial of Ovarian Cancer Screening) Longi-tudinal Women's Cohort UKLWC	England, Wales, Northern Ireland
Vorarlberg Health Monitoring and Promotion Programme (VHM&PP)	Austria
WHI (Women's Health Initiative)	USA

international collaborative research activities in the cancer epidemiology field in Japan. The field of cancer epidemiology has not only activated support for other countries where cancer statistics infrastructure is not well developed, but also large-scale compilation and international comparison through collaborative studies, and integration with analytical epidemiology and clinical research. Formation of international cohort consortia and estimates of cancer and risk factors in individual countries have not only contributed to improving the skills of cancer epidemiologists but also to expanding research networks and activities among them. Molecular and genome epidemiological studies on cancer have progressed over decades, and continue to do so in both size and dimension. Application of evidence from this area in prevention is still underway and requires further effort. Moving forward, Japanese epidemiologists have a major opportunity to take a leadership role in international collaborative research activities, especially in those focusing on major cancer types or exposure characteristics unique to the Japanese population.

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Management of frailty under COVID-19 pandemic in Japan

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Abstract: Frailty prevention is a cornerstone for the extension of healthy life expectancy. It is a multi-dimensional construct that includes physical, mental, and social aspects. Frailty is reversible and can be attenuated by intervention; therefore, its early detection is important in primary and community care. The mainstream of disability prevention in Japan, which comprises the Japanese and local governments as well as healthcare workers, was a high-risk approach until 2014. Given the revision on Japan's long-term care insurance law, current ageing health policies have shifted to more population centric approach. Implements group activities called "Kayoi-no-ba" has been valued in Japan as disability prevention initiative. The Kihon Checklist – a 25-item questionnaire – has been broadly used by health experts and researchers to assess frailty in Japan. However, a new 15-item questionnaire has been newly developed to identify frailty and other health-related problems in older people of 75 years and above. This will enable providing the necessary support to frail individuals at any healthcare facility in local communities. The increase in frailty prevalence in older people has been concern during the COVID-19 pandemic. Home-based physical exercise programs are expected to be effective for frailty prevention. Utilization of information and communication technologies, social network services, and video calls has attracted attention for being effective tools to facilitate communication for older people during the pandemic. Further, life course approaches are needed to clarify the midlife risk of frailty development in later life.

***Keywords*:** COVID-19, Kayoi-no-ba, HEPOP, community, social connection, ICT

Introduction

The number of elderly people worldwide is increasing rapidly, and the rate of this increase is fastest in Japan. While the average life expectancy is increasing year by year, improvements in healthy life expectancy have only tracked these in parallel (*1*) (Figure 1). The Japanese government has set a target of extending healthy life expectancy by three or more years by 2040 (*2*). To close the gap between the average life expectancy and healthy life expectancy, it is necessary for society as a whole to act against frailty.

In this article, we briefly review the definition and assessment of frailty and discuss how it can best be managed during the COVID-19 pandemic in Japan.

Definition of frailty

The word "frailty" has been attracting attention as a keyword in geriatric medicine and public health since the end of the 20th century (*3*). As a generic term, "frailty" is the noun form of frail, which means "easily broken or destroyed" or "physically weak." As a term in geriatric medicine and gerontology, experts involved in the Frailty Consensus Conference defined frailty in 2013 as follows (*4*): "A medical syndrome with multiple causes and contributors that is characterized by diminished strength, endurance, and reduced physiologic function that increases an individual's vulnerability for developing increased dependency and/or death". Similarly, the Japan Geriatrics Society defined frailty in 2014 as "a state of increased vulnerability to disability caused by various functional and/or intrinsic capacity deterioration due to aging" (*5*).

Among experts, the consensus is that frailty is reversible or attenuated by interventions; thus, early detection is important in primary and community care by health workers (*6*). Frailty is also characterized by multiple dimensions, namely, physical, mental, and social aspects, which are associated with each other and determine an individual's frailty status.

No standardized definition of frailty has been yet established (*4*), making it impossible to measure its prevalence, which is therefore uncertain. A systematic review of 21 studies including 61,500 older community residents revealed a wide variance in the prevalence of

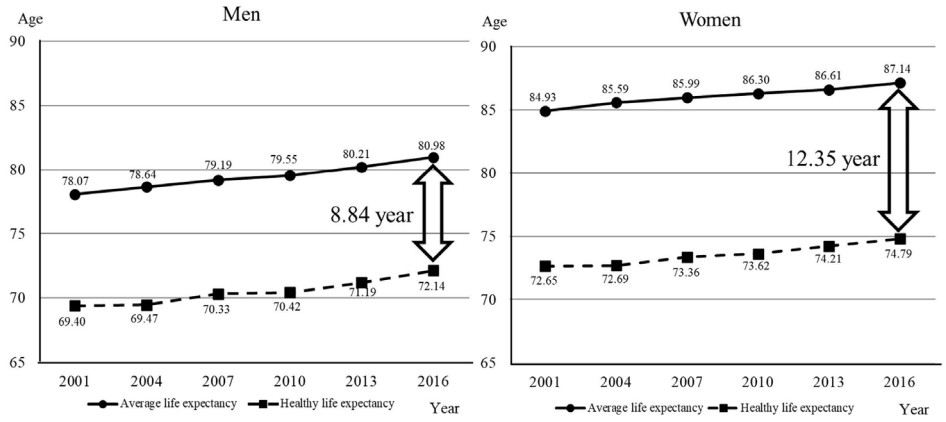

Figure 1. Chronological change of average life expectancy and healthy life expectancy. Average life expectancy is increasing year by year, but healthy life expectancy is only improving in parallel. Data source: Cabinet Office Japan. Annual Report on the Ageing Society 2019 (*1*).

frailty among studies (range 4.0-59.1%) and estimated the overall weighted prevalence as 11% (*7*). Frailty was generally found to be higher in women than in men, and it becomes more prevalent with age (*7-9*). Being a member of an ethnic minority group or having low socioeconomic status are considered risk factors for frailty (*8,9*).

There are two major models that have been widely used to operationalize the concept of physical frailty. One is the phenotype model proposed by Fried *et al.* (*10*), which suggested considering the presence of five symptoms to identify frailty in individuals: weight loss, exhaustion, weakness, slowness, and reduced physical activity. The criteria they developed for the Cardiovascular Health Study (CHS) has been widely used as the standard of physical frailty. Additionally, the deficit model proposed by Rockwood and Mitnitski accumulates the number of impairments and conditions to create frailty index (*11*).

With aging, muscle strength weakens, and this induces fatigue. Thus, older persons feeling excessive fatigue tend to avoid physical activity and stay home, which results in a further decline of muscle strength, energy expenditure, and appetite, thereby leading to weight and further muscle loss, that is, a frailty cycle (*10*). Fatigue and physical inactivity may cause sleep disturbance, depression, and social isolation, which accelerate the loss of appetite and vitality. There is no doubt that social disconnectedness, loneliness, and depression are associated with each other, and each increases the risk of physical frailty (*12*). However, there has been much debate about how to capture mental and social aspects of frailty (*13*).

Assessment of frailty

There have been a number of tools created to evaluate frailty. In their review article, Dent *et al.* listed 16 commonly used frailty instruments (*14*). It is important to choose an appropriate assessment tool for different types of patients (*15*). The validity of the instruments may vary by the setting and purpose, such as primary care facilities, hospitals, long-term care facilities, or local community facilities, where frailty prevention strategies have primarily been conducted by public health nurses.

As the standard criteria to diagnose frailty in Japan, the Japanese version of the CHS frailty index criteria (J-CHS criteria) was proposed based on the evidence of Japanese older adults (Table 1, J-CHS criteria) (*16*). According to the J-CHS criteria, prevalence of frailty among community dwellers ≥ 65 years ranged from 2.0% to 21.5% in Japan (*16*). Age-stratified meta-analyses showed the pooled prevalence of frailty defined by CHS criteria in Japan was 8.1% for women and 7.6% for men (*17*).

The J-CHS criteria include grip strength and gait speed, which requires actual measurement. Therefore, a more convenient tool is desired for broader application in public health practices. The Kihon Checklist (KCL) is a self-administrative questionnaire comprising 25 items (*18*), which was originally developed to select the targets for disability prevention by the Japanese Ministry of Health, Labour and Welfare (MHLW). It comprises seven domains, namely, instrumental ability of daily living, physical function, nutritional status, oral function, social isolation, cognitive function, and mental health. Its reliability and validity as a frailty screening tool against the CHS criteria has been demonstrated among outpatients (*19*). The validity of KCL as a predictive index of the need for long-term care and mortality among community-dwelling older people has also been confirmed (*20*). Furthermore, it has been translated into English, Spanish, Portuguese, and Simplified Chinese and has been widely used worldwide (*21*).

Recently, a new 15-item self-administrative questionnaire was developed and proposed by MHLW to assess health-related problems for older people, 75 years or above (*22,23*). It includes questions inquiring about general health perception; life satisfaction; and physical, cognitive, mental, social, and oral frailty. Detailed

Table 1. The J-CHS Criteria

Shrinking	Have you lost 2 kg or more in the past 6 months?	Yes = 1, No = 0
Low activity	1) Do you engage in moderate levels of physical exercise or sports aimed at health? 2) Do you engage in low levels of physical exercise aimed at health?	"No" to both questions = 1
Exhaustion	In the past 2 weeks have you felt tired without a reason?	Yes = 1, No = 0
Weakness	Grip strength Men: < 26 kg, Women: < 18 kg	
Slowness	Gait speed < 1.0 m/s	

Note: Frailty, prefrailty, and robustness are defined by having 3-5, 1-2, and 0 components, respectively (*13*).

Table 2. Questionnaire for medical checkup of the old-old (QMCOO) (*23*)

No.	Questions	Answer
1	How is your health?	① Excellent ② Good ③ Fair ④ Poor ⑤ Very poor
2	Are you satisfied with your daily life?	① Satisfied ② Moderately satisfied ③ Moderately dissatisfied ④ Dissatisfied
3	Do you eat three times a day?	① Yes ② No
4	Do you have any difficulties eating tough foods, compared to 6 months ago?	① Yes ② No
5	Have you choked on your tea or soup recently?	① Yes ② No
6	Have you lost 2 kg or more in the past 6 months?	① Yes ② No
7	Do you think you walk slower than before?	① Yes ② No
8	Have you experienced a fall in the past year?	① Yes ② No
9	Do you go for a walk for your health at least once a week?	① Yes ② No
10	Do your family or friends point out your memory loss? (*e.g.*, "You ask the same question over and over again.")	① Yes ② No
11	Do you find yourself not knowing today's date?	① Yes ② No
12	Do you smoke?	① Yes ② No ③ I quit
13	Do you go out at least once a week?	① Yes ② No
14	Do you keep up regular communication with your family and friends?	① Yes ② No
15	When you are not feeling well, do you have anyone you can talk to?	① Yes ② No

properties of the new frail questionnaire are under investigation. The recently developed Questionnaire for Medical Checkup of Old-Old (QMCOO) is designed to identify frail older persons in all healthcare settings, including at health checkups and in primary care clinics (Table 2) (*23*). This approach is expected to help frail patients find support that they need in their communities.

Management of frailty

Although the importance of the management of frailty is recognized globally, effective strategies have not been established yet (*14*). Physical activity, diet, and social participation are possibly the key components of an effective intervention because physical inactivity, malnutrition, and social isolation are considered as modifiable risk factors of frailty (*14*).

"Kayoi-no-ba" is a mutual focal point where locally living older residents can work on health promotion through physical exercise, hobbies or other activities, supported by local activists, while the whole activities and the process are supported by local government. The word for word translation of "Kaoyoi" is "commuting" and "ba" is "a place" in English. However, "ba" in this context implies a focal point where people with mutual interests are drawn to gather. As an intervening measure of frailty/disability prevention, "Kayoi-no-ba" generated greater public interests and is taken place across local communities in Japan. A large epidemiological study including 375,400 older adults aged 65 years or older living in a total of 81 regions (the Japan Gerontological Evaluation Study, JAGES, (*24*)) demonstrated that every social activity per hundred older people was significantly associated with an 11% reduction of the likelihood of frailty (odds ratio = 0.89; 95% credible interval = 0.81, 0.99) (*25*).

The mainstream of long-term care prevention in Japan used to be a high-risk approach, that is, efforts were made to identify and support individuals whose ability to perform activities of daily living was declining. Because of the revision to the Long-Term Care Insurance Law in 2014, the policy was shifted to more population-centric approach. "Kayoi-no-ba" came to the forefront regarding the measures for frailty and disability prevention. Both the number of "Kayoi-no-ba" and the prevalence of participants have kept increasing. According to a government report, there were 106,766 places and 5.7% of the community-dwelling older people participated in "Kayoi-no-ba" in 2018 (*26*). In 2019, the government announced an 8% increase to the participation rate of "Kayoi-no-ba" by 2025 as a numerical target of an initiative to prevent dementia.

From April 2020, "Integration of long-term care prevention and health services for the elderly" has been promoted by the MHLW. In this initiative, health professionals—such as public health nurses, registered

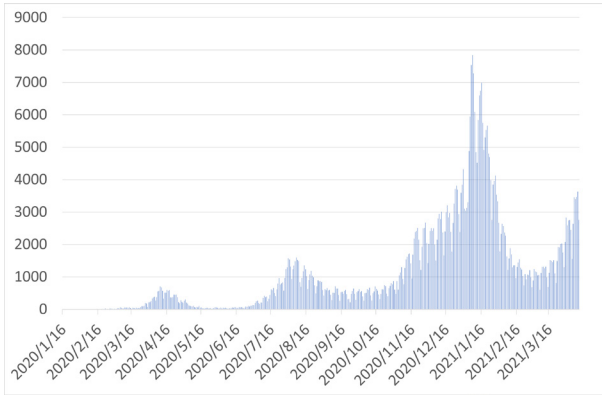

Figure 2. Number of daily confirmed cases of COVID-19 tested by PCR in Japan. During the period of January 16, 2020 and April 12, 2021. Data source: Ministry of Health, Labour and Welfare, Open data (*28*).

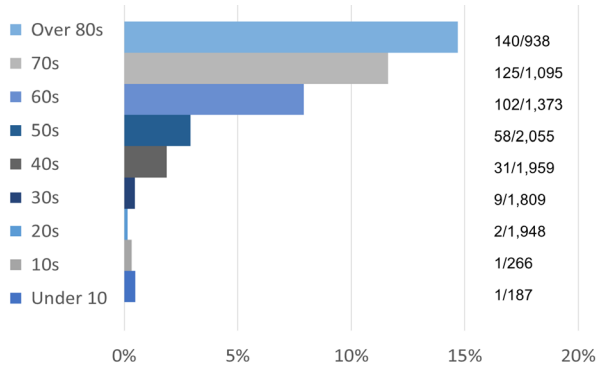

Figure 3. Prevalence of severe cases among confirmed cases of COVID-19 during the first wave of COVID-19 pandemic in Japan. Older people were more likely to become seriously ill from suffering from COVID-19 during the period of January 16 and April 22, 2020, in the first wave of COVID-19 pandemic. Data source: Ministry of Health, Labour and Welfare, The case of coronavirus, April 23, 2020 (*29*).

dieticians, dental hygienists, physical therapists, occupational therapists, and speech therapists – are expected to actively get involved with "Kayoi-no-ba" by providing health consultation and education to the participants. They are also expected to evaluate participants' health condition and identify frail persons by using QMCOO, the new 15-item questionnaire for frailty assessment (*23*) to direct them toward the necessary support and medical services.

In the implementation of integrated disability prevention and healthcare services for older people, one of the major tasks is utilizing the database of national health insurance (Kokumin Hoken Database, KDB) to organize and analyze the health issues of local communities. Local municipalities are responsible for the provision of healthcare services to the residents. KDB is linked to the databases for long-term care services, so that it enables provision of all information regarding medical and long-term care for each older person.

Health professionals who visit "Kayoi-no-ba" can evaluate the health condition of older individuals by using QMCOO, along with information on health checkups, medical care, and long-term care extracted from the KDB system. Then, they would advise the older person based on the available data and introduce them to appropriate healthcare services. Furthermore, by recording individuals' participation in "Kayoi-no-ba" in the KDB data, it is possible to evaluate the effectiveness of the "Kayoi-no-ba" as a frailty prevention strategy in the targeted area. From now on, by monitoring the effectiveness of "Kayoi-no-ba," it is expected to further improve the quality of the "Kayoi-no-ba" program in accordance with the plan-do-check-act cycle of local community-based integrated care system (*27*).

The COVID-19 pandemic and frailty

COVID-19, which began in Wuhan, China, started spreading throughout the world sometime in late 2019. The WHO declared it as a pandemic on March 11, 2020.

Figure 2 shows the daily number of reported COVID-19 cases in Japan during the period of January 16, 2020 and April 12, 2021 (*28*). In Japan, the first cases were reported on January 16, 2020, and hit its first peak in mid-April, with the number of new cases reaching 720 a day. From early April to May, a state of emergency was declared; movie theaters and department stores were closed, and people were asked to refrain from going out. The number of infected people rose again, reaching a second peak of 1,604 at the beginning of August. It began to increase in early November with a peak of 7,844 on January 8. A fourth wave came in early April, 2021, and the number of cases including new variants continues to increase even now (April 19, 2021).

One of the characteristics of COVID-19 is that older people are more likely to become seriously ill. In the first wave, older people were the major victims of the COVID-19 pandemic (Figure 3) (*29*). Most seniors refrained from going out for fear of infection and stayed home.

It is obvious that restraint from going out will accelerate the frailty cycle in older people. The impact of the COVID-19 pandemic on the development of frailty has only been beginning to emerge. Thus, we need to consider what can and should be done for better management of frailty during the pandemic (*30,31*).

Prevention of frailty under the COVID-19 pandemic: NCGG-HEPOP

At time of writing (mid-April 2021), it is unpredictable when the COVID-19 pandemic will come to an end. For older adults, staying at home for fear of infection is considered to be a risk in terms of the development of frailty. Thus, efforts to prevent frailty along with infection control are needed (*32,33*).

Presently, efforts for frailty prevention during the COVID-19 pandemic are being made. The National Center of Geriatrics and Gerontology (NCGG) has

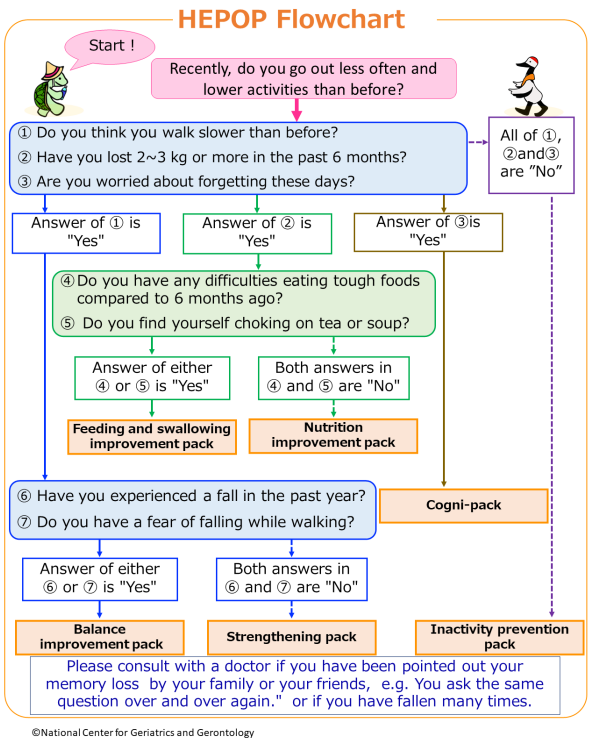

Figure 4. HEPOP flowchart. Reproduced from (*34*) by permission of National Center for Geriatrics and Gerontology. Those who would like to start HEPOP, the home exercise program, can use the flowchart to determine which program package is the most appropriate for them. The questionnaire items are derived from Kihon Checklist (*18*) and QMCOO (*23*).

developed a guide for home-based activities for frailty prevention and made it available on the web (*34*) (*https://www.ncgg.go.jp/hospital/english/hepop/*). The guide for Home Exercise Program for Older People (HEPOP) introduces six packages, including physical exercises, eating methods, and cognitive training, that older individuals can practice at home according to their individual abilities or needs in an easy-to-understand manner. The packages include balance improvement, physical strengthening, inactivity prevention, cognitive, feeding and swallowing improvement, and nutrition improvement.

Those who would like to start HEPOP, the home exercise program, can use the flowchart to determine which package is the most appropriate (Figure 4). As the appropriateness of the package may change depending on the patient's physical and mental status, it is recommended to reassess once a month or whenever any change is perceived. Before starting an exercise or activity, it is recommended to check the "precautions and methods of use" and "exercise intensity" to select the most appropriate exercise or activity for each individual's needs.

The balance improvement package is for frail or pre-frail individuals who have fear of falling to improve their muscle strength and balancing ability. The physical strengthening package is for those who perceive slowness in their walking speed, and it is designed to improve and maintain physical functions and strength. The inactivity prevention package is designed to help robust older people to maintain their physical functions. All three packages include stretching, balance, muscle training, and whole-body exercises. These combine various exercises and require 40 to 60 minutes per day depending on the self-condition (20 to 60 minutes for the balance improvement package). The cogni-package is for those who are afraid of cognitive decline. It includes several tasks combining "exercise" with "cognition," which we call "cognicise" (*35*): dual-task training, conducting cognitive tasks such as word games, performing fast simple numerical calculations, and playing a simple memory span game while performing aerobic exercises. The feeding and swallowing improvement package is for those who have lost more than 2 to 3 kg of weight during the past six months and feel difficulties in eating and/or swallowing and who want to be provided with basic information about oral frailty (*36*) and exercises to maintain oral and swallowing function. The nutrition improvement package is for those who lost weight without oral frailty and is designed to provide information about malnutrition and advice to improve their diet.

HEPOP is applicable to all older people with stable physical condition and no serious pain, including inpatients on discharge, outpatients, and older people living in the community. Implementation, adoption, and effectiveness of HEPOP are under investigation.

Social connection under the COVID-19 pandemic

The COVID-19 pandemic forces older people to avoid physical contact with others, which increases psychological distress and reduces sources of support, thus leading them to frailty or more severe consequences. To maintain social connection during the COVID-19 pandemic is the most difficult part of frailty prevention, especially for older people living alone. While phone calls and postal letters are common conventional communication tools, e-mails have become popular. Use of social network services and video calls has attracted attention as effective tools during the pandemic to facilitate communication for older people (*33*). According to reports from the Ministry of Internal Affairs and Communications (*37*), 90.5% of 60-year-olds, 74.2% of 70-year-olds, and 57.5% of 80-year-olds were using internet in 2019. Although the evidence regarding the effectiveness of video call in the reduction of loneliness in older adults are still limited (*38*), intervention utilizing information and communication technologies (ICT) has been promoted. Note that ICT is not always beneficial for all people (*39*), and promoting ICT may increase inequality in terms of access and usage because not everyone can afford to buy ICT tools or have enough time and skills to keep up with the latest information as well as reach out to various support services. Those

who are disadvantaged could be left far behind in this information age. Thus, it is crucial to provide sufficient support for older people to use ICT such as mobile phones or computers as well as other options.

Future implication

Recently, Taniguchi *et al.* examined 2,675 Japanese people aged 65-90 years prospectively and identified four distinct trajectory patterns (*40*). According to their report, 36.3% had very high functional capacity at the age of 65 and maintained higher functional capacity even after 85 years of age. In contrast, 6.1% had low functional capacity at the age of 65 already and kept losing their functional capacity year by year. The rest shared the same level of functional capacity at the age of 65 years; however, 40.1% maintained the level until 85 years of age and 17.4% saw a decrease. Their study implies that most of those who would be frail in the future may have been already determined at the age of 65. This is consistent with the findings of the Whitehall II study by Brunner *et al.* that health behaviors and biomedical risk factors measured at 50 years of age and above explained more than one-third of socioeconomic inequalities in frailty (*41*).

It is essential to detect frail individual among older persons to provide them with the necessary support. It is also important to intervene with middle-aged people to limit future frailty. More life course studies are needed to clarify the modifiable risk factors concerning frailty.

Conclusion

There is an urgent need to establish an effective frailty prevention strategy for use during the COVID-19 pandemic. A newly developed 15-item questionnaire and home-based physical exercise programs are expected to promote frailty management in Japan. Life course approaches are also needed to clarify the midlife risk for frailty development in later life.

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Promoting analysis of real-world data: Prospects for preventive cardiology in Japan

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Abstract: According to the statistics for 2018 in Japan, cardiovascular disease and cerebrovascular disease were the most common causes of death (cardiovascular disease with 208,210, cerebrovascular disease with 108,165), and these two diseases account for 23.2% of all deaths. Stroke, especially cerebral hemorrhage, was the main cause of death in Japan after World War II. Along with improved management of hypertension, the mortality rate from cerebral hemorrhage reached a high of 266.7 per 100,000 men in 1960 and 213.9 per 100,000 women in 1951, then decreased to 15.9 per 100,000 men and 6.9 per 100,000 women in 2013. However, mortality from lifestyle-related diseases such as metabolic syndrome and ischemic heart disease has been on the rise since 1990 due to the westernization of diet, urban lifestyles, and lack of exercise habits. Moreover, since aging is the greatest risk factor for heart failure, the number of patients with heart failure in Japan will inevitably increase in the future. A large amount of evidence has demonstrated that prevention and proper management of risk factors can reduce the future incidence of cardiovascular disease. Specific health checkups (metabolic syndrome checkups) have been carried out in Japan since 2008. Big data on physical examinations are valuable real-world data that can be utilized for clinical research. As the importance of preventive cardiology increases in the future, we should analyze the real-world data from health checkups in Japan in detail and disseminate these results to clinical practice, which will contribute to development of preventive cardiology and the promotion of public health.

***Keywords*:** preventive medicine, cardiovascular disease, modifiable risk factors, health checkup

Introduction

In January 2020, the Cabinet Office announced the "Moonshot Goals" (*1*). The Moonshot Goals define six specific challenges that are difficult, but which, if realized, will have a significant impact on society. One of them is to "create a society in which it is possible to predict and prevent disease at an extremely early stage by 2050". Preventive medicine is now a national project of great importance in Japan.

The purpose of this review is to overview the efforts of preventive medicine in cardiovascular diseases (CVD). We will consider the importance of CVD in the medical care of Japan and the efforts of preventive cardiology in comparison with the situation in other countries.

The place of cardiovascular disease in the history of medicine

The most recent causes of deaths in Japan (statistics for 2018) were as follows: malignant neoplasms (tumors) with 373,547, CVD with 208,210, senility with 109,606, and cerebrovascular disease with 108,165. CVD and cerebrovascular disease account for 23.2% of all deaths (*2*).

According to the United States statistics for 2018, the leading causes of the 2,813,503 deaths per year in the United States are heart disease (647,457), malignant neoplasms (599,108), accidents (169,936), chronic respiratory diseases (160,201), and strokes (146,383). CVD account for 28.2% of all deaths, including heart disease and stroke (*3*). Conquering CVD is a challenge not only for Japan, but for most developed countries.

The place of CVD in the history of medicine has largely changed. Today, the novel coronavirus infection (COVID-19) is rampant throughout the world. Since the dawn of history, infectious diseases have been the greatest threat to humanity. The Black Death in the 14th and 16th centuries and the Spanish flu in the early 20th century are the most notable examples of infectious disease pandemics. In Japan, tuberculosis was the leading cause of death in the early 20th century (the death rate from tuberculosis in 1918 was 257.1 per 100,000 people, far higher than that of malignant neoplasms (*4*)). On the

other hand, prevention of infectious diseases was greatly promoted by improved sanitary conditions and the development of vaccines or antibiotics. The Tuberculosis Prevention Act was enacted in 1951, and the prevalence of streptomycin and other antimicrobial treatments has dramatically reduced the mortality rate due to tuberculosis in Japan.

While deaths from tuberculosis have decreased dramatically, stroke, particularly cerebral hemorrhage, was the main cause of death in Japan after World War II. The high salt content of the Japanese diet originally caused a high prevalence of hypertension, and the lack of proper control of hypertension is thought to have increased the number of deaths from cerebral hemorrhage. A national campaign to reduce salt intake was launched, and the Labor Standards Law required employers to provide workers with medical examinations in 1947, and the amendments to the National Health Insurance Law made antihypertensive treatment more widespread in 1961, which improved the management of hypertension. Along with improved management of hypertension, the mortality rate from stroke (cerebral hemorrhage) has dramatically decreased (Figure 1). The mortality rate from cerebral hemorrhage reached a high of 266.7 per 100,000 men in 1960 and 213.9 per 100,000 women in 1951, then decreased to 15.9 per 100,000 men and 6.9 per 100,000 women in 2013 (*5*). In 1972, the Occupational Health and Safety Law was enacted, and in addition to the tuberculosis-related health checkup items that had been included in the Labor Standards Law, items such as blood pressure measurement were added to the health checkup. Since then, items aimed at health management other than countermeasures against infectious diseases have been added, and these items are now part of regular health checkups. Under the Community Health Law, health centers have been established throughout the country to provide health checkups for local residents (*6*).

While deaths from cerebral hemorrhage have decreased, mortality from lifestyle-related diseases such as metabolic syndrome and ischemic heart disease has been on the rise since 1990 due to unhealthy lifestyles including the westernization of diet, urban lifestyles, and physical inactivity (Figure 2). To reduce the burden of ischemic heart disease, it is important to prevent these lifestyle-related diseases that are increasing in middle-aged adults.

In 2008, the Ministry of Health, Labour and Welfare (MHLW) started the Specific Health Examination (commonly known as "metabolic syndrome health examination") (*7*) for adults between the ages of 40 and 74. The Specific Health Examination is a rare initiative in the world that aims for early detection of lifestyle-related diseases such as metabolic syndrome and the prevention of CVD on a nationwide scale. Ten years have already passed since the Specific Health Examination began, and it is now time to review the effectiveness of the Specific Health Examination and devise more effective ways to conduct health examinations.

Heart failure is one of the most important CVD that will become increasingly important in the future. The number of patients with heart failure has been increasing rapidly in Japan due to the rapid aging of society and the increase in the number of young and middle-aged people with ischemic heart disease. The number of patients with heart failure is estimated to continue to rise, reaching 1.3 million by 2030 in Japan (*8*).

The epidemiological situation of an increasing number of patients with heart failure is called "heart failure pandemic", which is a common problem not only in Japan but also in other developed countries with aging populations. In 2015, the proportion of the elderly

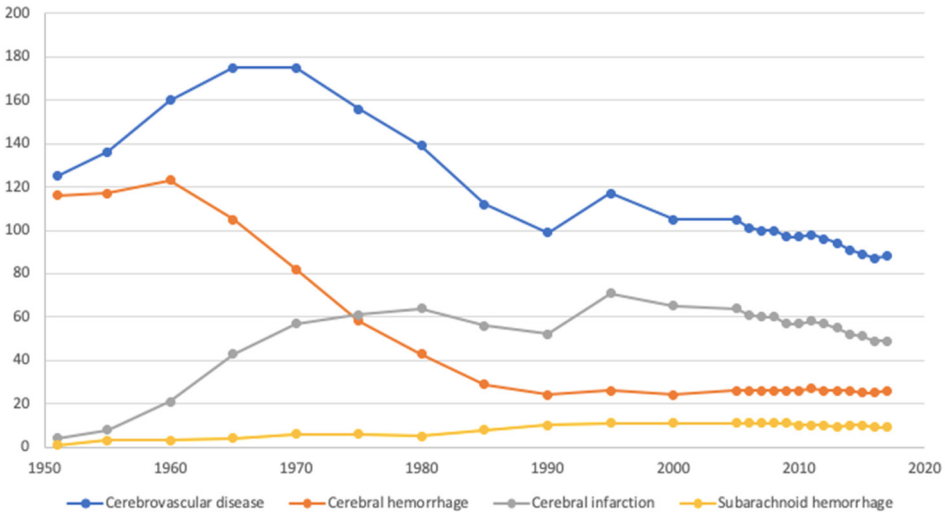

Figure 1. Trends in mortality by stroke category in Japan (per 100,000). Data Source: (*5*).

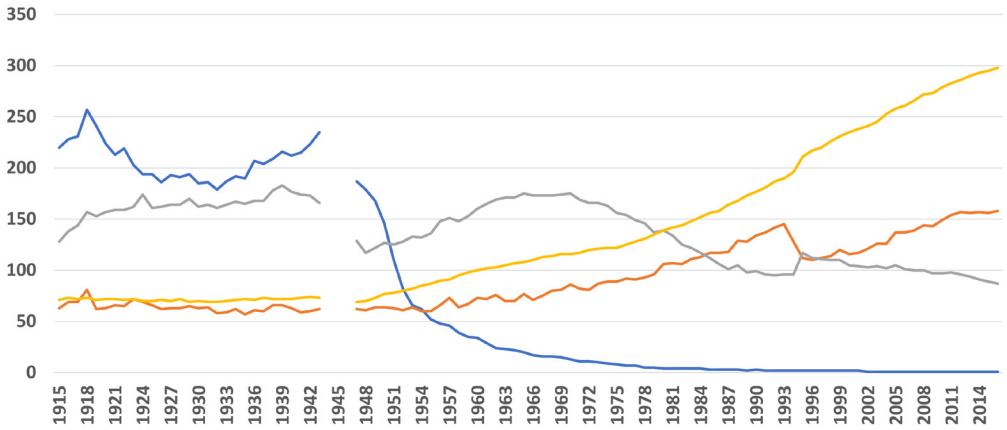

Figure 2. Mortality rate by disease (per 100,000). Blue: tuberculosis; gray: cerebrovascular disease; orange: ischemic heart disease; yellow: malignant neoplasm. Data Source: (*4*).

in Japan (the percentage of the population aged 65 and over) was 26.6%, which is higher compared to 14.6% in the United States, 21.1% in Germany, and 9.7% in China (*9*). Japan is one of the countries with the most aged population in the world. Japan is aging rapidly: in 1960, Japan's aging rate was 5.7%, whereas in 2010 it reached 23.0% (Figure S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=27*). In 2018, for a population of 126.43 million, there were 35.56 million (28.1%) people aged 65 years and older, and 17.95 million (14.2%) people in the later stages of 75 years and older. Declining birthrate as well as aging population in Japan is a difficult problem to solve, and the percentage of aged population is expected to continue to increase in the future, with the Cabinet Office estimating that by 2065, approximately 1 in 2.6 Japanese will be 65 years of age or older, and 1 in 3.9 will be 75 years of age or older (*10*). Because aging is the greatest risk factor for heart failure, the number of patients with heart failure in Japan will inevitably increase in the future. In our study, more than 50% of heart failure patients were aged 80 years or older, and the number of very old people aged 90 years or older was close to 20% of heart failure patients (*11*). As an advanced aging society, Japan's efforts to address the heart failure pandemic at the national level are getting attention from countries around the world.

Three reasons why prevention is important in cardiovascular disease

Effect on healthy life expectancy

Although Japan is known as one of the countries with the longest life expectancy in the world, there is a gap of about 10 years between healthy life expectancy and actual life expectancy (about 9 years for men and 12 years for women (*12*)). This gap is a period of time during which patients are limited in their daily lives due to health problems, such as the need for support and care, which not only degrades the quality of life of the individual patient, but also places a heavy burden on the family to support the patient, which cannot be overlooked in terms of social productivity. The fact that CVD, including stroke and heart failure, are major causes of reduced healthy life expectancy (*13-15*) as well as reduced life expectancy, is one of the major reasons why it is important to prevent CVD.

Impact on medical costs

The cost of medical care in Japan has continued to increase: from 16.0 trillion yen (160 billion dollars) in 1985 to 30.1 trillion yen (301 billion dollars) in 2000 and 42.3 trillion yen (423 billion dollars) in 2015 (Figure S2, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=27*). The share of medical costs for the elderly in the later stages of life has also increased as a percentage of national medical costs, rising from 25.4% in 1985 to 35.9% in 2015 (*16*). CVD are a significant burden on medical costs, with medical care costs for CVD of 5.9 trillion yen (59 billion dollars), which accounted for the highest percentage (19.9%), followed by "Malignant neoplasms" at 4,125.7 billion yen (41.2 billion dollars) (13.7%), "Musculoskeletal and connective tissue diseases" at 2,326.1 billion yen (23.2 billion dollars) (7.7%), "Respiratory diseases" at 2,223.0 billion yen (22.2 billion dollars) (7.4%), and "Injury, poisoning and other external causes" at 221.2 billion yen (2.2 billion dollars) (7.4%) (*17*). By age group, malignant neoplasms accounted for 1,521.2 billion yen (15.2 billion dollars) (13.0% of the total) more than CVD among those under 65 years of age, but CVD accounted for 4,686.9 billion yen (46.9 billion dollars) (25.5%) more than malignant neoplasms among those 65 years of age and older. Considering that Japan's population will be aging further in the future, prevention of CVD is extremely important from a health care economic standpoint.

Prevention is effective for cardiovascular disease

Many epidemiological studies have identified a variety of risk factors for CVD. As discussed in more detail below, many of the risk factors for CVD are modifiable risk factors that can be ameliorated by lifestyle modifications and pharmacological intervention, and a large body of evidence demonstrates that proper management of these modifiable risk factors can reduce the future incidence of CVD (*18-20*). In Japan, a major objective of widespread health screening is to detect these risk factors early. The major goal of widespread health screening in Japan is the early detection of these risk factors and early intervention to prevent the development of CVD. It should be emphasized that, compared with malignancies, degenerative diseases, and genetic disorders, modifying risk factors is more effective for the prevention of most CVD.

Epidemiological studies identifying risk factors for cardiovascular disease

Many epidemiological studies have been conducted in Japan and abroad to clarify risk factors for CVD. Representative epidemiological studies are described below.

Framingham study

The Framingham study (*21*), the first large-scale epidemiological study of CVD, was initiated in 1948 by the National Institutes of Health in Framingham, Massachusetts, USA. At the time, the increase in CVD was an epidemiologic problem in the United States, but little was known about risk factors of CVD. The Framingham study, which began with 5,209 adult residents between the ages of 30 and 62, is an historic study that is now on its third generation of participants. The Framingham study revealed risk factors for CVD, including obesity, hypertension, hypercholesterolemia, diabetes mellitus, smoking, and physical inactivity (*22*), which have shaped today's cardiovascular practice. Framingham Risk Score, which is widely used in the CVD prediction, was published in 1998. Framingham Risk Score predicts incidence of ischemic heart disease within 10 years based on the sum of age, gender, total cholesterol, high-density lipoprotein (HDL) cholesterol, systolic blood pressure, blood pressure medication, and smoking status (*23*).

Hisayama study

Since 1961, the Department of Public Health of Kyushu University has been leading a prospective follow-up study of CVD which mainly focused on stroke among the residents of Hisayama-cho (population 8,400), which is adjacent to Fukuoka City (*24*). Residents of Hisayama have about the same age and occupational distribution as the national average, and they represent an average Japanese population with little or no bias. The study was prompted by the fact that, at the time, deaths from cerebral hemorrhage were 12.4 times higher than those from cerebral infarction in Japan, which was significantly higher than in Europe and the United States, and there were inquiries from European and American researchers that the predominance of cerebral hemorrhage may have been due to a misdiagnosis. There was no data to verify this, so the Hisayama study was initiated to clarify the reality of stroke in Japan. Early data of Hisayama study showed that the mortality rate from cerebral hemorrhage was 1.1 times that of cerebral infarction, which was inconsistent with the Japanese report at that time, which stated that the mortality rate from cerebral hemorrhage was 12.4 times that of cerebral infarction. Therefore, it is possible that some of the cases diagnosed as cerebral hemorrhage should have been diagnosed as cerebral infarction. A unique feature of the Hisayama study is the high autopsy rate of 75%. Autopsies are indispensable in determining the exact cause of death. The follow-up rate is also high (99%), and a new cohort of residents aged 40 years or older is added to the study every 5 years, providing insight into changes in lifestyle and prevalence of risk factors.

Although there is an amount of knowledge that has been revealed by the Hisayama study, we highlight two of them here. First, high diurnal variation in home blood pressure was found to be a risk for development of dementia (*25*). Although observational studies had previously shown that high variation in blood pressure per reception was indicative of cognitive decline, none had evaluated home blood pressure. This study suggested that home blood pressure variability and hypertension were associated with the development of vascular dementia (*25*), whereas home blood pressure variability (independent of hypertension) was a risk for Alzheimer's disease (*26*). In another study, airflow limitation was found to be a significant risk factor for carotid atherosclerosis, especially in middle age (*27*). This is the first study to show a relationship between chronic obstructive pulmonary disease with airflow limitation and carotid atherosclerosis in a large group of the Asian population (*27*).

Suita study

This cohort study began in 1989. Of 12,200 men and women randomly selected from the basic resident register in Suita, Osaka Prefecture, 6,485 people aged 30-79 years old who underwent an initial health examination were included in the study (*28*). After the initial health check-up, the participants undergo regular check-ups every two years at the National Cerebral and Cardiovascular Center. The survey is conducted annually

by mail or telephone. The Suita Cardiovascular Disease Score, which predicts the probability of developing coronary artery disease and stroke within 10 years, was developed based on data tracking the incidence of coronary artery disease and stroke through December 2013 (*29*).

The risk of myocardial infarction in the Japanese population is enormously lower than that in Westerners. A comparison of the diagnostic performance of the Framingham Risk Score, a score used in Europe and the United States and the Suita score showed that the Framingham Risk Score tended to overestimate the probability of developing coronary artery disease (up to 14%), whereas the Suita score almost accurately predicted the probability of developing coronary artery disease (*30*). The Suita score is expected to be useful in predicting cardiovascular events in the general population living in an urban setting.

Modifiable risk factors in cardiovascular disease

Obesity and metabolic syndrome

Medical problems from a nutritional perspective have shifted from being caused by malnutrition to overnutrition in the immediate aftermath of World War II. The diet of the Japanese people changed dramatically between 1950 and 1975, with intake of meat, poultry and eggs increasing 7.5-fold, fat intake increasing 6-fold, and milk intake increasing 15-fold (*31*). Westernization of dietary habits and overnutrition led to a noticeable increase in obese subjects. As shown in the Figure, the average BMI has increased, especially among men (Figure 3A), and the proportion of obesity has increased accordingly. Because obesity is closely related to the development of lifestyle-related diseases and even CVD (*32*), early detection of overweight and obesity through health checkups and other measures, including nutritional and exercise guidance, are necessary to maintain an appropriate body weight.

The diagnostic criteria for metabolic syndrome in Japan include *i*) Waist circumference of ≥ 85 cm for men and ≥ 90 cm for women; *ii*) Two or more of the following items must be met: (a) triglycerides ≥ 150 mg/dL, or HDL < 40 mg/dL, (b) systolic blood pressure of ≥ 130 mmHg or diastolic blood pressure of ≥ 85 mmHg, (c) fasting blood glucose of ≥110 mg/dL (*33*).

Obesity, abdominal obesity (high waist circumference), and metabolic syndrome often coexist. On the other hand, the term "metabolically healthy obesity" has been used to describe a condition that is obese but not associated with metabolic disorders or the metabolic syndrome, and it has received much attention (*34,35*). There are two definitions of obesity by itself, one with a cutoff of BMI 30 kg/m^2 and the other with a cutoff of BMI 25 kg/m^2. Furthermore, metabolically healthy obesity is often defined as a condition in which "obesity is present but no metabolic abnormalities are present at all," and is sometimes defined as "being obese but not meeting the definition of metabolic syndrome." The fact that the definition of metabolic syndrome differs from country to country is another reason for the confusion in the definition of metabolically healthy obesity. In addition, there are cases of metabolic syndrome that shift from metabolically healthy obesity to metabolically unhealthy obesity over time, and more detailed studies in this area will help to establish the definition and understand the optimal management for "metabolically healthy obesity".

Hypertension

Although it is important to exclude secondary hypertension, such as primary hyperaldosteronism, from hypertensive cases, many of the cases have so-called "essential" hypertension, which is caused by a combination of environmental and genetic factors that contribute to the predisposition to hypertension. Epidemiological studies in Japan have shown a correlation between average salt intake and the prevalence of hypertension in the region. Epidemiological studies have also shown that dietary treatment, such as salt restriction, is effective in lowering blood pressure. Conventional Japanese diets contain too much salt, which increases the prevalence of hypertension and contributes to the incidence of stroke. As mentioned above, national-scale interventions in salt intake restriction and hypertension management began around 1960, and the daily salt intake of Japanese people was about 15 g in the 1950s, but in the 2010s it was less than 10 g (Figure 3B) (*36*). Salt reduction is important not only for the prevention of hypertension, but also for the prevention of heart failure and chronic renal failure, and we should continue efforts to reduce salt intake.

A current topic in hypertension is the diagnostic criteria for hypertension: the ACC/AHA blood pressure guidelines published in 2017 lowered the value of blood pressure in the diagnostic criteria for hypertension from 140 mmHg of systolic blood pressure and 90 mmHg of diastolic blood pressure to 130 mmHg of systolic blood pressure and 80 mmHg of diastolic blood pressure (*37*). On the other hand, the European guidelines remained the previous definition of hypertension and defined hypertension as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg (*38*). Similarly, the Japanese guidelines published in 2019 did not change the criteria for hypertension, and therefore, the diagnostic value for hypertension is the systolic blood pressure of 140 mmHg or the diastolic blood pressure of 90 mmHg (*39*). On the other hand, as the SPRINT study (*40*) showed, strict antihypertensive treatment has proven to be effective in reducing cardiovascular events. In addition, large registry studies

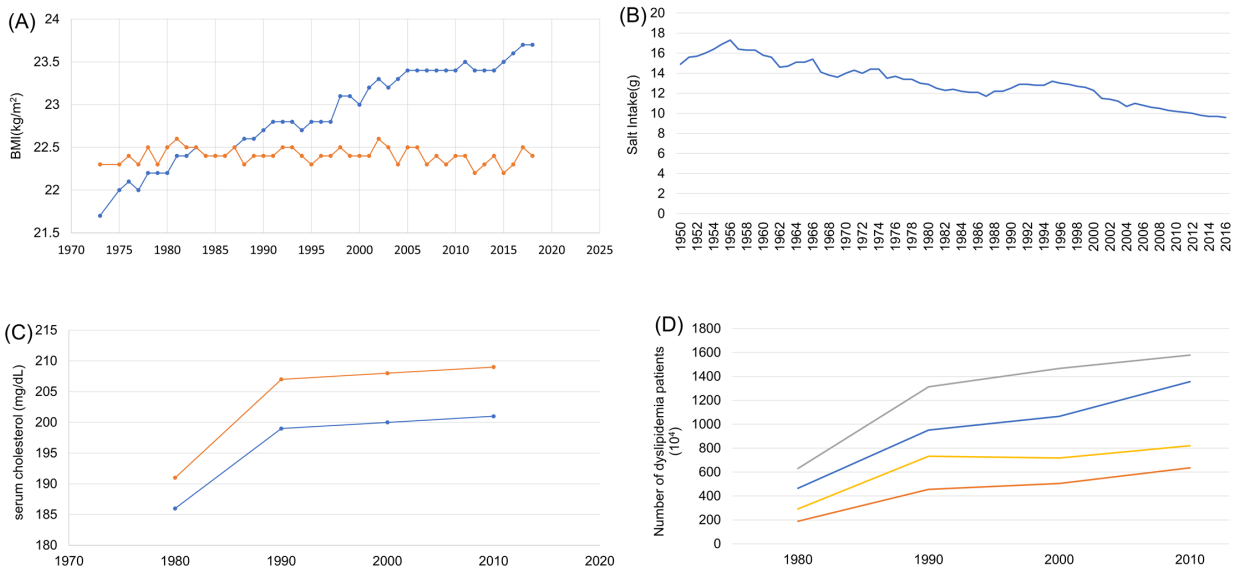

Figure 3. Trends in modifiable risk factors of cardiovascular disease among Japanese. (A) Trends in BMI. Blue: male; Orange: female. **(B)** Trends in salt intake. **(C)** Trends in serum cholesterol levels (mg/dL). Blue: male; Orange: female. **(D)** Prevalence of dyslipidemia, age 30 years and older. Blue: > 220 mg/dL (male); orange: > 240 mg/dL (male); gray: > 220 mg/dL (female); yellow: > 240 mg/dL (female). Data Source: (*https://www.nibiohn.go.jp/eiken/kenkounippon21/eiyouchousa/keinen_henka_shintai.html* (*36, 44*)).

in the United States and South Korea have shown that blood pressure classifications based on the 2017 ACC/AHA blood pressure guidelines are effective for risk stratification of cardiovascular events in young adults (*41,42*). Furthermore, the analysis of a population-database in Japan also showed that compared with normal blood pressure, both stage 1 hypertension and stage 2 hypertension according to the 2017 ACC/AHA blood pressure guidelines were associated with an elevated risk of heart failure and atrial fibrillation (*43*). We need further data to establish the cut-off value of blood pressure for the diagnosis of hypertension and to determine the optimal target value of blood pressure for the prevention of CVD.

Hypercholesterolemia (Dyslipidemia)

The prevalence of hypercholesterolemia and dyslipidemia in the Japanese population has changed significantly due to the westernization of diet in the Japanese people. The earliest report on lipid levels in official statistics is the Third Cardiovascular Disease Survey, conducted in 1980 (*44*). The Cardiovascular Disease Survey is conducted every 10 years, and it reports the change in lipid levels for the 30 years from 1980 to 2010. The increase in serum cholesterol levels between 1980 and 1990 for both men and women was greater than in the latter two decades, from 186 mg/dL in 1980 to 199 mg/dL in 1990 for men aged 30 years and older, and from 191 mg/dL to 207 mg/dL for women aged 30 years and older, with an increase of 13-16 mg/dL in 10 years, respectively (Figure 3C). The prevalence of dyslipidemia in people aged 30 years and older changed from 1980 to 2010 in the Cardiovascular Disease Basic Survey and the National Health and Nutrition Survey (Figure 3D). These results were estimated from the prevalence of hypercholesterolemia based on the census population and total cholesterol levels in each year. The criteria for hypercholesterolemia which have long been used in Japan and the United States are as follows: 220 mg/dL or higher in Japan and 240 mg/dL or higher in the United States (*44*).

In addition, according to a 2017 survey by the Ministry of Health, Labour and Welfare on the proportion of people with suspected dyslipidemia according to age and sex, the proportion of men is higher than that of women under 60 years of age, whereas the proportion of women is similar to that of men over 60 years of age (Table 1) (*45*).

For patients with hypercholesterolemia (dyslipidemia), it is important to first investigate the causes (*e.g.* familial hypercholesterolemia, hypothyroidism), as well as to provide nutritional and lifestyle guidance. We believe that early introduction of statins is the key to prevent future CVD. Statins have become an essential part of the armamentarium against CVD based on the extensive evidence both on primary prevention and secondary prevention for CVD. As shown in the Table, the lower the low-density lipoprotein cholesterol (LDL-C) levels achieved in both primary and secondary CVD prevention, the lower the incidence of cardiovascular event rate (Table 2) (*46*). Particularly for secondary CVD prevention, the LDL-C target of less

Table 1. Percentage of people with suspected dyslipidemia by age and gender

	Total	20-29	30-39	40-49	50-59	60-69	70~
Total	42.2	21.1	26.3	35.4	52.2	52.3	43.9
Male	47.7	27.0	44.2	52.8	56.7	52.6	41.4
Female	38.6	17.2	16.9	25.9	49.3	52.0	45.9

Table 2. Relationship between LDL-C achievement and cardiovascular event rates

	Trial	Baseline LDL-C (mg/dL)	LCL-C Achieved (mg/dL)	Statin Event Rate (%)	Placebo Event Rate (%)
Primary prevention	WOSCOPS	192	159	5.3	7.5
	AFCAPS	150	115	3.5	5.5
Secondary prevention	4S	188	122	19.4	27.9
	CARE	139	98	10.2	13.2
	LIPID	150	112	12.3	15.7

than 70 mg/dL should be achieved. However, achieving the therapeutic target of LDL-C is sometimes difficult in real-world clinical practice. Notably, there may be cases that require the highest dose of statins, multiple drugs, or even PCSK9 inhibitors, particularly in cases of familial hypercholesterolemia.

Diabetes mellitus

It is also widely known that diabetes mellitus is associated with CVD. Although non-pharmacological treatments such as dietary counseling are the first choice for diabetes mellitus, in practice, many patients require pharmacological treatment including oral diabetic drugs or insulin. The current topic in the treatment of diabetes mellitus is the SGLT2 inhibitors, which exert their glucose-lowering effects by inhibiting glucose reuptake in the renal proximal tubules. Large clinical trials such as the ACCORD trial (*47*), the ADVANCE trial (*48*) and the VADT trial (*49*) have shown that strict glycemic control does not reduce cardiovascular events in type 2 diabetes mellitus. In addition, DPP4 inhibitors, which have shown positive results in animal studies, did not reduce cardiovascular events in the SAVOR-TIMI53 (*50*), EXAMINE (*51*), and TECOS studies (*52*). Rather, the SAVOR study showed an increase in heart failure hospitalizations in the DPP4 inhibitor group. In the EMPAREG-OUTCOME study (*53*) and the CANVAS study (*54*), SGLT2 inhibitors were shown to reduce cardiovascular events (*55*), and have become the focus of attention in diabetes mellitus treatment. In particular, the effect of SGLT2 inhibitors on heart failure events is an important finding in the heart failure pandemic era arriving in the near future. The subsequent DAPA-HF study (*56*) showed that SGLT2 inhibitors reduce cardiovascular death and heart failure hospitalizations in heart failure patients with reduced left ventricular systolic function, with or without diabetes mellitus. The mechanism of this beneficial effect of SGLT2 inhibitors is still unclear, and further studies are needed to understand the mechanism of this cardiovascular effect.

Smoking

Smoking is also a major risk for CVD. Smoking increases the risk of mortality from CVD by 2.07-fold (*57*). Our study also showed that smoking would increase unstable plaque in the carotid arteries (*58*). Passive smoking, even in the absence of smoking, has also been shown to increase the risk of atherosclerosis, stroke, and all-cause mortality. A meta-analysis shows that smoking increases all-cause mortality by 1.18 times and CVD by 1.23 times (*59*). In addition, one-third of coronary artery disease deaths were associated with smoking or passive smoking (*32*). In Japan, awareness-raising efforts have been vigorous and smoking rates have decreased significantly (Figure S3, ,https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=27) (*60*). Because smoking is also a risk factor for the development of many malignancies, additional anti-smoking educational activities are needed.

Physical inactivity

Lack of exercise habits has also been shown to be a risk factor for CVD (*32*). The development of urban lifestyles, including sedentary work indoors, has reduced the amount of time people spend on exercise and physical activity. An important theme in preventive medicine is the setting of appropriate exercise habits tailored to each person's lifestyle.

Japan's health examination system and comparison with other countries

We have described that CVD are important in Japan's medical care in various ways, and that early detection and treatment of lifestyle-related diseases underlying CVD can prevent development of CVD. Japan's health checkup system would be useful for early detection of

lifestyle-related diseases and prevention of future CVD.

The health examination system in Japan has three main components: health examination of workers, health examination of community residents, and health examination of students. The first health examination of workers was made mandatory by employers under the Labor Standards Law, which was enacted in 1947. At that time, the main purpose was the early detection of tuberculosis, but after that, with a decrease in the number of tuberculosis infections. The Occupational Safety and Health Law was enacted in 1972, and blood pressure measurement was added for the purpose of early detection and treatment of hypertension. Since then, health checkups have been launched for the purpose of health management, and these have become the current regular health checkups. Health checkups for local residents are carried out by health centers under the Community Health Law. Students are required to undergo medical examinations by June 30 of each school year under the School Health and Safety Act of 1958.

In addition, the system of voluntary physical examinations called "Ningen (Human) dock", which began in 1954, is unique to Japan. This system was initiated for the wealthy, but it has since become more widespread, with approximately three million people undergoing the procedure each year. The physical examinations also include options such as brain and heart checks, and the early detection of CVD is one of the main purposes of the complete physical examinations.

In the United States, health examinations are also conducted under the direction of family physicians and are voluntary, as in Japan, but the content of the examinations is not as uniform as in Japan. In the U.K., free medical examinations are provided by the national health insurance system through registered family physicians, but the content of these examinations is not as comprehensive as in Japan.

In this regard, it is of academic interest to know the extent to which the specific health checkups (metabolic syndrome checkups) that have been carried out in Japan since 2008, as well as the Ningen dock for 3 million people who voluntarily undergo them at their own expense, contribute to the early detection and prevention of lifestyle-related diseases and CVD.

Utilizing physical examination data and health checkup data for clinical research

A large amount of data on physical examinations are valuable real-world data that can be utilized for clinical research. In the following, we would like to introduce our research activities using these real-world data.

Physical examination data analyses have already shown various findings. These below are several examples of our research in collaboration with the Department of Cardiovascular Medicine and the Preventive Medicine Center, the University of Tokyo Hospital. The definition of hypertension differs among the United States and Europe and Japan, which is controversial even today. The association between carotid intima-media thickening, a good predictor of future CVD, and the new BP categories according to updated ACC/AHA guidelines was examined in those who underwent physical examinations at the Preventive Medicine Center. The results showed that, in stage 1 hypertension, which is defined as systolic blood pressure of 130-139 mmHg or diastolic blood pressure of 80-89 mmHg, early phase atherosclerotic plaques were more likely to develop than in the normotensive group (*61*). Similar results were obtained for CAVI (cardio-ankle vascular index), a measure of arterial stiffness, in males (*62*). These findings suggest that stage 1 hypertension according to the updated ACC/AHA blood pressure classification is significantly associated with atherosclerotic change in adults.

Another example is about body weight change, which is one of the most important parameters in preventive medicine. We reported that body composition data showed that body fat weight change accounts for about 70% of the body weight change in adulthood (*63*), and that this should be considered to be primarily a change in fat weight, both during weight gain and during weight loss. It was also reported that changes in body weight over time are strongly associated with changes in blood pressure (*64*) and lipid profiles (*65*), emphasizing the importance of weight control in management of lifestyle-related diseases.

We want to mention metabolically healthy obesity, which is defined as "obesity (BMI $\geq$ 25 kg/m^2) but not meeting the diagnostic criteria for metabolic syndrome in Japan" compared to the non-obese group. It has been shown that carotid intima-media thickness progresses in the metabolically healthy obesity group (*66*). On the other hand, the risk of atherosclerosis increases from the stage when abdominal circumference is smaller than the criteria for abdominal circumference (85 cm or more for men and 90 cm or more for women), which is included in the diagnostic criteria for metabolic syndrome in Japan (*67*), suggesting the optimal abdominal circumference cutoff for predicting development of cardiovascular events remains controversial. Smoking was shown to be an independent risk factor for carotid artery high-risk plaque formation (*58*), suggesting that smoking is significantly related to the early-phase pathogenic process of acute coronary syndromes and cerebral infarction.

With regard to health screening, we are collaborating with the Department of Clinical Epidemiology on a study using the JMDC Claims Database, which mainly collects data on health checkups and insurance claims data. It has already shown that the Cardiovascular Health Metrics, an indicator of risk for CVD, are correlated between couples (*68*), and that a weight gain

of 10 kg or more from age 20 years, even in the absence of obesity, is associated with an increased risk of future cardiovascular events (*69*). Additionally, we have also reported the importance of modifiable risk factors for the prevention of CVD in young adults (*70-72*), and the association of the blood pressure classification according to the 2017 ACC/AHA guideline with the incidence of CVD (*43*). These real-world data will have great potential for further clinical investigation.

As the importance of preventive cardiology increases in the future, we should analyze the real-world data from health checkups in Japan in detail and disseminate these results to clinical practice, which would promote preventive medicine and public health.

Conclusion

By reviewing the changes in the major causes of death in Japan, we believe that CVD will become even more important in Japan in the future, and that they will not be overlooked in terms of shortening healthy life expectancy and increasing medical costs. Most importantly, CVD can be prevented by intervening in the modifiable lifestyle and lifestyle-related diseases that predispose people to CVD. In light of these points, Japan's universal health insurance system and extensive health checkup system, which cover almost all of its citizens, would play an important role. We need to disseminate knowledge gained by use of data from the universal health insurance system, and the health checkup system conducted on the Japanese people, which will contribute to the development of preventive cardiology.

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Current status and future perspectives of onco-cardiology: Importance of early detection and intervention for cardiotoxicity, and cardiovascular complication of novel cancer treatment

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Abstract: The prognosis has improved remarkably in recent years with the development of cancer treatment. With the increase in the number of cancer survivors, complications of cardiovascular disease have become a problem. Therefore, the field of onco-cardiology has been attracting attention. The field of onco-cardiology covers a wide range of areas. In the past, cardiac dysfunction caused by cardiotoxic drug therapies such as doxorubicin (Adriamycin) was the most common cause of cardiac dysfunction, but nowadays, cardiovascular complications caused by aging cancer survivors, atherosclerotic disease in cardiovascular risk carriers, thromboembolism, and new drugs (*e.g.*, myocarditis caused by immune checkpoint inhibitors and hypertension caused by angiogenesis) are becoming more common. In this review, we summarize the latest findings of cardiotoxicity of cancer therapy, appropriate treatment and prevention, and cardiovascular complications of novel chemotherapy, which will increase in demand in the near future.

***Keywords*:** cardiotoxicity, anthracycline, immune checkpoint inhibitors-associated myocarditis, cancer-associated thrombosis (CAT), radiotherapy

Introduction

Because treatment for cancer has dramatically developed and prognosis has improved, cancer survivors have recently increased. While cancer is the leading cause of death, complications with the second leading cardiac disease are on the rise, and the need for management of cardiovascular complications in cancer survivors is increasing. Cancer treatment also has its characteristic side effects, and cardiovascular complications can be severe (*1*). Improvements in cancer have also led to an aging population of cancer patients and an increase in the number of cancer patients with cardiovascular risk. Now that reintegration into society after living with cancer is commonplace, there is a need to avoid the development of cardiovascular complications and interruption of cancer treatment. The relationship between cancer treatment and cardiovascular diseases/complications is receiving more and more attention, and the collaboration between oncologists and cardiologists is becoming more and more important.

The field of onco-cardiology was first featured in the 1960s when anthracyclines were used as a new cancer treatment. In the United States, the world's first onco-cardiology unit was established at MD Anderson in 2000. Barac *et al.* have presented the following roadmap (Figure 1) for the oncology and cardiology unit (*2*). Onco-cardiologists need to work seamlessly with cancer survivors, not only after cancer-associated heart disease has occurred, but also during the prevention phase of cancer development, treatment, and after cancer treatment has been completed.

In this review, we summarize the latest findings of cardiotoxicity for cancer therapy, appropriate treatment and prevention, and cardiovascular complications of novel chemotherapy, which will increase in demand in the near future.

Cardiac dysfunction and cardiovascular complications of chemotherapeutic drugs

Anthracycline-induced cardiotoxicity has been the most well known cardiac problem associated with cancer treatment. However, with the advent of molecularly targeted therapy and other advances in cancer treatment, new cancer treatment-related cardiovascular diseases have been reported. In addition to heart failure due to reduced cardiac function, vascular thromboembolism, such as myocardial infarction, and immune checkpoint inhibitor (ICI) -associated myocarditis have also been reported (*3*).

Cardiac dysfunction

Definitions of cardiac dysfunction

The most common cancer treatment-related cardiotoxicity is cardiac dysfunction resulting in heart failure. It is defined by the position paper from Europe Society of Cardiology (ESC) as the following: a decrease in left ventricular ejection fraction (LVEF) of at least 10% and LVEF less than 55% (*4*). The American Society of Echocardiography (ASE) has also defined the following: an LVEF of at least 10 percentage points below baseline and an LVEF of 53% or less (*5*), American Society of Clinical Oncology (ASCO) (*6*) and European Society of Medical Oncology (ESMO) (*7*) have also their own definitions (Table 1).

Cardiotoxicity from cancer treatment became of interest in the 1960s when anthracycline-induced cardiomyopathy was found as a fatal complication. In terms of whether cardiac dysfunction is reversible or not, they have been classically classified as Type 1 and Type 2 (*4,8*). Type 1 is dose-dependent and causes irreversible histological changes, as typified by anthracyclines; Type 2 is dose-independent and reversible. However, this classification is no longer used because drugs that are known to be reversible with type 2 are also irreversible in about 20% of cases (*8*). The latest guidelines of ASCO do not refer to them as Type I or II since the resulting cardiotoxicity/ myocardial dysfunction is more important than the mechanism of cardiotoxicity as a drug (*6*). Recently, tyrosine kinase inhibitors (*9*) and proteasome inhibitors (*10*) have also been reported to cause cardiomyopathy.

Clinical features and pathophysiology

For early detection of cardiovascular disease in cancer patients and the cardiovascular complications associated with cancer treatment, it is important to assess the patient's risk of cardiovascular complications prior to treatment. The traditional cardiovascular risk factors such as self-monitoring blood pressure, HbA1c and low-density lipoprotein (LDL) cholesterol levels, electrocardiography (ECG), and chest x-ray are important for this purpose. Patients at high risk need careful follow-up.

Careful daily physical examination is also important for early detection. The complaint of shortness of breath on exertion and chest pain, the findings of leg edema, and weight gain is checked at every visit. An

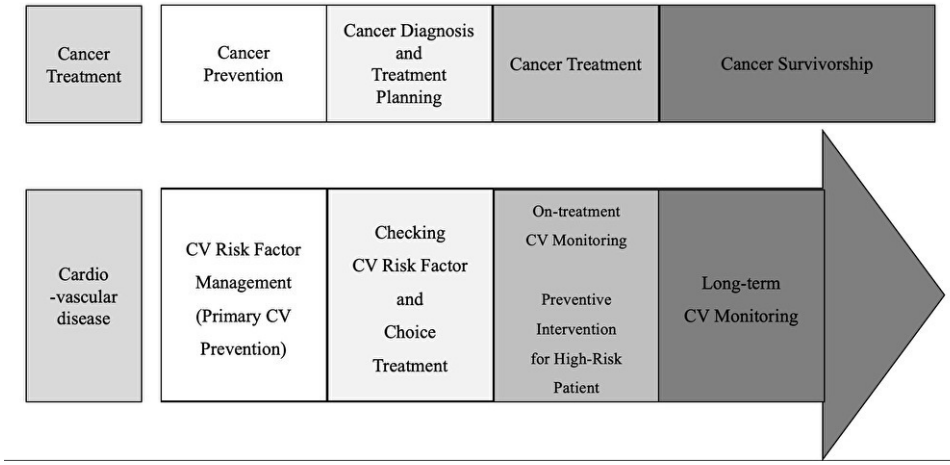

Figure 1. The roadmap for following the oncology and cardiology unit (*2*). Onco-cardiologists need to work seamlessly with cancer survivors, not only after cancer-associated heart disease has occurred, but also during the prevention phase of cancer development, treatment, and after cancer treatment has been completed.

Table 1. The definition of cancer treatment-related cardiotoxicity

	ASCO (*6*) Clinical practice guideline	ESMO (*7*) Clinical guideline	EACVI/ASE (*5*) Expert consensus	ESC (*4*) Position paper
Definition	An asymptomatic decrease in LVEF of 10% to less than 55% or a symptomatic decrease in LVEF of 5% to less than 55%	Decline in LVEF of at least 5% to less than 55% with accompanying signs or symptoms of congestive heart failure, or a decline in LVEF of at least 10% to below 55% without accompanying signs or symtoms	Decrease in the LVEF of > 10% points, to a value < 53%	LVEF: > 10% points decrease to a value below the lower limit of normality GLS: 15% relative percentage reduction from baseline

ASCO: American Society of Clinical Oncology; ASE: American Society of Echocardiography; EACVI: European Association for Cardiovascular Imaging; ESC: European Society of Cardiology; ESMO: European Society for Medical Oncology; GLS: Global Longitudinal Strain; LVEF: Left Ventricular Ejection Fraction.

arrhythmia is also checked by listening to the heartbeat and palpating the pulse. The abnormalities of ECG such as low voltage QRS potentials, ST changes, and QT prolongation may reflect cardiac dysfunction at a relatively early stage. It is still important to compare the results with those before treatment. A number of novel tyrosine kinase inhibitors, which have been used in cancer treatment, may cause QT prolongation and ECG should be checked (*11,12*). A chest x-ray is also used to check the cardiothoracic ratio and pleural effusion. Echocardiography is a very important and useful test. In general, decreased LVEF is an important finding, but is not suitable for early detection. The speckle tracking method used in echocardiography is a method that automatically tracks fine myocardial speckles using pattern matching to obtain coordinates (*13*). In particular, the left ventricular global long strain (GLS) obtained on the apical long-axis image is highly reproducible and can be useful for early diagnosis of disease and may be a prognostic factor (*5,14,15*). In high-risk patients, follow-up every 3 months is desirable (*16*). Either BNP or NT-proBNP in plasma may be useful as a biomarker for heart failure. Both BNP and NT-proBNP are elevated in advanced cardiac load and also useful in determining the response to treatment for cardiotoxicity (*4*). Troponin is a myocardial-specific marker that reflects myocardial damage and myocardial necrosis. Elevated troponin levels after the start of cancer treatment is predictive of a decrease of EF (*17*) and have been reported to be predictive of events such as cardiac death and heart failure (*18*). In any case, specific blood tests are recommended before cancer treatment and regularly when using potentially cardiotoxic drugs.

Anthracyclines

Anthracyclines act on nucleic acids regardless of what stage of the cell cycle and exert anti-tumor effects through DNA intercalation and inhibiting topoisomerase type II. Topoisomerase II is responsible for cleaving two strands of DNA. Doxorubicin forms a complex with topoisomerase type II and DNA expressed in cardiomyocytes, and the retention of DNA double-strand breaks cause cardiotoxicity (*19*). It is also reported to cause damage to the vascular endothelium (*20*). The frequency of doxorubicin increases with cumulative lifetime dose of 400 mg/m^2. For example, at a cumulative dose of 400 mg/m^2, the incidence of congestive heart failure is 5%, but above 700 mg/m^2, the incidence exceeds 48% (*21*).

The ASCO guidelines specify a high risk of cardiac dysfunction with anthracyclines use, and a high-risk group has been reported to have a cardiotoxicity risk even at doses below 250 mg/m^2 (*6*). The high-risk groups include the following: high-dose anthracyclines (> 250 mg/m^2), high-dose radiation (30 Gy) involving the heart in the field, having 2 or more cardiovascular risk factors such as smoking, hypertension, diabetes mellitus, dyslipidemia, or obesity, older age (> 60 years at the time of cancer treatment), decreasing cardiac function before or during treatment (LVEF50-55%, previous myocardial infarction, moderate or severe valvular disease), and sequential therapy with trastuzumab. Lyon *et al.* also stratified risk by history of cardiac disease, biomarkers, and risk of coronary disease (*22*). The time of onset of anthracycline cardiotoxicity has historically been classified as acute, early, and late (*23*). However, 98% of cases of cardiotoxicity now occur within the first year (*24*).

In particular, GLS on echocardiography is reported to detect cardiotoxicity at an early stage and should be checked frequently (*25*). Cardiac magnetic resonance imaging (CMR) has also been reported to provide early detection, especially T2 mapping, which is reported to be the earliest marker (*26,27*). It has also been reported that elevated troponin after the initiation of anthracyclines predicts left ventricular dysfunction and cardiac events (*17*).

HER2 inhibitors

HER2 is a receptor tyrosine kinase, which is involved in the regulation of cell proliferation, differentiation, migration, and survival (*28*). It is also involved in the development and maintenance of the nerves of the heart. In particular, trastuzumab is a key drug in HER2-positive breast cancer, and this drug has improved cancer prognosis (*29,30*). Trastuzumab is thought to increase the cardiotoxicity of anthracyclines, and Slamon *et al.* reported symptomatic or asymptomatic cardiac dysfunction was found in 27% of patients with the combination of anthracyclines and trastuzumab (*29*). In a 10-year follow-up of HER2-positive breast cancer, impaired cardiac function was 9.4% in the group assigned to a regimen containing paclitaxel, cyclophosphamide, and trastuzumab and 19.2% in the group assigned to an anthracycline-containing regimen (*31*). With paclitaxel plus trastuzumab, as the standard of care for HER2-positive early-stage breast cancer, heart failure is relatively rare, with Grade 3 or greater symptomatic heart failure reported in 0.5% and cardiac dysfunction in 3.2% of patients (*32*). The cardiotoxicity of trastuzumab is said to be reversible, but about one-third is reported to be prolonged (*8*). Pertuzumab is essentially a drug used in combination with trastuzumab; the Food and Drug Administration (FDA) recommends checking cardiac function every 3 months during treatment for recurrence and every 6 weeks for neoadjuvant chemotherapy. Lapatinib is used in combination with capecitabine in metastatic recurrent breast cancer. The incidence of cardiac events with lapatinib was as low as 1.6%, and mostly asymptomatic, with only a decrease in EF. Asymptomatic events were as low as 0.2% (*33*).

A pooled analysis (n = 1,961) of trastuzumab emtansine (T-DM1) showed some cardiac dysfunction

in 3.4% of patients (*34*). Trastuzumab-delux-Tecan caused 0.9% of patients to have impaired cardiac function (*35*). However, none of these patients have had much experience with the drug. The patients were already on trastuzumab for second-line use, and the use of anthracyclines should also be noted.

Vascular endothelial growth factor Inhibitors

Vascular endothelial growth factor (VEGF) has been identified as a factor that increases vascular permeability and causes angiogenesis (*36*). VEGF inhibitors improve the reach of antitumor drugs to cancer by blocking excessive VEGF signaling and normalizing tumor blood vessels, which are also highly permeable. VEGF inhibitors include the monoclonal antibodies bevacizumab and ramucirumab, as well as several other multi-kinase inhibitors that are used to treat a variety of cancers. VEGF is also thought to be important for tissue growth and angiogenesis in the heart; inhibition of VEGF is known to inhibit cardiac remodeling and cause heart failure. The most frequent cardiotoxicity is hypertension, but cardiac dysfunction is less common. In a large prospective observational study of breast cancer patients, bevacizumab caused left ventricular dysfunction in 2% of patients and symptomatic heart failure in 1% (*37*). With sunitinib, pazopanib, and axitinib, cardiac dysfunction was reported in 3 to 15% and symptomatic heart failure in 1 to 10% of patients (*4*). Risk factors include coronary artery disease, a history of valvular disease, and a history of anthracycline use.

Other tyrosine kinase inhibitors

The advent of tyrosine kinase inhibitors has dramatically improved the prognosis of chronic myeloid leukemia (*38*). For the cardiovascular system, ischemic heart disease and pulmonary hypertension have been reported as serious complications (*9*). There are few reports of cardiotoxicity with imatinib (*39*).

Treatment and prevention for cardiac dysfunction

Felker *et al.* reported that anthracycline-induced cardiomyopathy had a very poor prognosis, with a 2-year survival rate of 40% (*40*). A recent single-center report showed that with precise monitoring and appropriate early treatment when it occurred, the prognosis was no different than that of hereditary cardiomyopathy (*41*).

If symptomatic heart failure develops during chemotherapy, discontinuation of current chemotherapy should be considered. According to current heart failure guidelines, cardioprotective therapy such as using angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), and beta-blockers, has been recommended, but there is no established evidence (*4,42*). The European Society for Medical Oncology (ESMO) guidelines for cardiac dysfunction caused by chemotherapy indicate a different response for type 1 and type 2 (*43*).

Only small reports of primary prevention have also been reported. In ACE inhibitors, patients with elevated troponin I immediately after initiation of high-dose adriamycin treatment divided into ACE inhibitor and non-ACE inhibitor groups, the ACE inhibitor group prevented the development of late cardiotoxicity (*44*).

In the beta-blocker group, Kalay *et al.* reported that prophylaxis by carvedilol prevented cardiac dysfunction in a small group of patients receiving anthracyclines (*45*). Avila *et al.* also reported that prophylaxis by carvedilol in patients scheduled to receive adriamycin prevented elevated troponin levels and diastolic dysfunction but did not change LVEF (*46*).

Some reports indicate an anti-inflammatory preventive effect of statins used for hyperlipidemia; Seicean *et al.* reported an association between statin use and the development of heart failure, although in a retrospective study (*47*). Although Vaduganathan *et al.* published a meta-analysis that the use of beta-blockers, ACE inhibitors, and ARBs was effective in primary prevention, heterogeneity and publication bias were noted and there still has been only limited evidence for these effects (*48*).

Dexrazoxane is a topoisomerase II beta inhibitor, intracellular iron chelator, and increases hypoxia-inducible transcription factors. It is expected to inhibit cardiomyocyte cell death and apoptosis, which is the basis for the development of cardiotoxicity, and has potential for a long-term protective effect against cardiotoxicity of anthracycline (*49*). Kalam *et al.* conducted a meta-analysis of 14 articles on prevention of heart failure, which also showed the efficacy of dexrazoxane, beta-blockers, ACE inhibitors, and statins (*50*). Further large, prospective, randomized trials are awaited (Table 2).

Immune checkpoint inhibitor (ICI) -associated myocarditis

ICIs are anti-tumor drugs with a new mechanism of action: by binding to immune checkpoint molecules such as PD-1 and CTLA-4, as well as to their co-receptor-binding ligands, they block inhibitory signals and enhance the immune response against cancer.

Since Brahmer *et al.* reported efficacy in non-small cell lung cancer (*51*), various trials have shown improved outcomes with the use of immune checkpoint inhibitors and in combination with other agents. The use of immune checkpoint inhibitors has been found to have characteristic side effects, such as the development or exacerbation of autoimmune and inflammatory diseases due to the loss of normal immune regulation (*52*). These immune-mediated side effects are called immune-related adverse events (irAEs). Cardiovascular complications such as vasculitis, arrhythmias,

Table 2. The summary of clinical studies of prevention of cardiotoxicity

Drug	Authors, Year (*Ref.*)	Type of Study	*n*	Target Group	Dose	Control Group	Follow-up Period	Outcome	Result (%)	*p*-value or Risk Ratio [95% CI]
ACE inhibitor	Cardinale, *et al.* 2006 (*44*)	RCT	56 *vs.* 58	Patients who showed a troponin I increase soon after high dose chemotherapy	Enalapril 20 mg/day	Not receiving ACE inhibitor	12 months	Absolute decrease > 10% units in rest LVEF associated with a decline below 50%	0 (0) *vs.* 25 (43)	< 0.001
Beta Blocker	Kalay N, *et al.* 2006 (*45*)	RCT	25/25	Patients whom antracycline therapy was planned	Carvedilol 12.5 mg/day	Placebo	6 months	LVEF	Carvedilol: 70.5 *vs.* 69.7 Placebo: 68.9 *vs.* 52.3 (baseline *vs.* follow)	Carvedilol: $p = 0.3$ Placebo: $p < 0.001$
Beta Blocker	Avila MS, *et al.* 2017 (*46*)	RCT	96/96	Patients with HER2-negative breast cancer tumor status and normal LVEF referred for anthracycline (240 mg/m^2)	Beginning with a dose of 3.125 mg twice a day, which was increased to 6.25 mg, then to a maximum dose of 25 mg	Placebo	6 months	Prevention of a ≥ 10% reduction in LVEF	14 (14.5) *vs.* 13 (13.5)	1.0
Dexrazoxane	Macedo AVS, *et al.* 2019 (*49*)	Meta-analysis	575/605	Patients with breast cancer receiving anthracycline with or without trastuzumab	1000 mg/m^2 or 10:1 or 20:1 (DEX : DOX) dose ratio	No therapy or Placebo	126 days to 5 years	Clinical heart failure	RR 0.19 [0.09 to 0.40]	< 0.001
Dexrazoxane	Macedo AVS, *et al.* 2019 (*49*)	Meta-analysis	383/1,414	Patients with breast cancer receiving anthracycline with or without trastuzumab	1000 mg/m^2 or 10:1 or 20:1 (DEX : DOX) dose ratio	No therapy or Placebo	126 days to 5 years	Cardiac events (Subclinical heart failure or admission due to cardiac causes)	RR 0.36 [0.27 to 0.49]	< 0.001

ACE: Angiotensin Converting Enzyme; CI: Confidence Interval; DEX: Dexrazoxane; DOX: Doxorubicin; LVEF: Left Ventricular Ejection Fraction; RCT: Randomized Control Trial; RR: Risk Ratio.

takotsubo-like syndrome, and pericardial disease have been reported, but the most common is autoimmune myocarditis (*53*). Johnson *et al.* reported a case of ICI-associated myocarditis in patients treated with nivolumab in combination with ipilimumab 12 and 15 days after treatment (*54*). Mahmood *et al.* reported that myocarditis occurred in 1.14% of patients at a median of 34 days and was more common in the combination group and in diabetes mellitus (*55*). Electrocardiographic changes were elevated in 89% of patients and troponin was elevated in 94% of cases. Hu *et al.* recommend checking baseline ECG and troponin before treatment and following up with troponin measurement every 4-6 weeks. If abnormalities are found, they recommend immediate ICI withdrawal and close examination, including echocardiography and CMR (*53*).

As for treatment, early initiation of high-dose steroids has been reported to improve prognosis (*53,55*). Zhang *et al.* reported an improved prognosis with early steroid pulse therapy in a retrospective study of 126 cases of myocarditis (*56*). Other high-dose therapies such as mycophenolate, infliximab, and anti-thymocyte globulin have been recommended as third-line treatment for steroid-refractory patients (*57*). In some cases of steroid refractory patients, abatacept, a CTLA-4 agonist, has been reported to be effective (*58*), but further clinical data are needed.

Ischemic heart disease

Ischemic heart disease (IHD) is the second leading cause of death after cancer. The number of cancer survivors is on the rise, as is the number of patients with cancer who are at risk for coronary artery disease due to an aging population. Factors such as smoking, diabetes, and obesity are also common risk factors for cancer patients and atherosclerotic atherothrombosis. Navi *et al.* reported a 2-3 times greater risk of myocardial infarction and cerebral infarction at 6 months after cancer diagnosis in cancer patients and non-cancer patients (*59*). With regard to therapeutic agents, 5-FU, widely used for gastrointestinal and gynecological cancers, had a 7.6% incidence of cardiovascular events and a 2.2% mortality rate at high doses (*60*). The mechanism may also involve endothelial damage and vasospasm (*61*). Cisplatin is an alkylating agent used in lung and gastrointestinal cancers. IHD is the most serious vascular hazard of cisplatin. The concomitant use of 5-FU increases the risk. The incidence of arterial thrombosis in patients treated with cisplatin-based chemotherapy was approximately 2% (*62*).

In recent years, particular attention has been paid to IHD caused by angiogenesis inhibitors such as anti-VEGF antibodies. The incidence of myocardial infarction has been reported to be 3.8% with bevacizumab (*4*). In a meta-analysis of 77 phase III RCTs, compared with non-users, arterial thrombosis (OR 1.52 [95% CI 1.17-1.98]), cardiac dysfunction (OR 1.35 [95% CI 1.06-1.70]), and myocardial ischemia (OR 2.83 [95% CI 1.72-4.65]) were found in the angiogenesis inhibitor group with VEGF inhibitors. Endothelial damage is thought to cause vasospasm and atherosclerotic collapse (*63*).

Hypertension

It is one of the most frequent problems in cancer treatment-related vascular disorders. Hypertension caused by VEGF inhibitors and multi-kinase inhibitors is typical. The frequency has been reported to be 11-45% (*4,64,65*). In a previous meta-analysis, VEGF inhibitor-induced hypertension was reported to have an OR of 5.28 [NNH 6] and severe hypertension to have an OR of 5.59 [NNH 17] (*66*). The mechanism in the acute phase is thought to be NO pathway inhibition by VEGF pathway inhibition and associated vasoconstriction (*67*). In the chronic phase, a decrease in vascular bed size and an associated increase in vascular resistance are thought to be the cause. Guideline-based drug treatment of hypertension is fundamental. When using VEGF inhibitors, regular weekly monitoring during the first cycle of treatment is preferred. Thereafter, monitoring every 2-3 weeks during VEGF inhibitor use is necessary (*68*). Because hypertension has also been reported to be a predictor of treatment response with bevacizumab (*69*) and sunitinib (*70*), proper management of hypertension and avoidance of discontinuation is preferred. Other anti-androgenic and antiestrogenic drugs for prostate cancer can also increase blood pressure.

Arrhythmia

There are a wide variety of arrhythmias that can be caused by anti-tumor treatment (*4,71*). However, data on incidence and causation are scarce. This is because the arrhythmia itself may not be identified if it is transient or asymptomatic. Because of the possibility of fatal arrhythmias, the accumulation of data and the development of high-quality evidence are needed.

QT prolongation

QT prolongation syndrome can result in fatal arrhythmias such as Torsade de pointes (TdP). During chemotherapy, electrolyte abnormalities due to vomiting and diarrhea, especially hypokalemia, may be caused by antiemetic and antipsychotic drugs. It can also be exacerbated by women, the elderly, the presence of underlying cardiac disease, and subclinical congenital long QT syndrome (LQTS) (*4*). Arsenic trioxide, used for treatment of leukemia and myeloma, is a typical agent that causes QT prolongation in 26-93%. In 40% of cases, QT interval is greater than 500 msec and occurs between 1-5 weeks after administration (*72*). Tyrosine kinase

inhibitors also cause QT prolongation. The incidence varies from drug to drug, but is relatively small with vandetanib, lapatinib and others (*4,12*).

ECG and electrolytes are necessary prior to treatment, and ECG is recommended for 7 to 15 days and monthly for the first 3 months after drug administration and change of dose (*4*). When QT prolongation is observed, treatment should be temporarily interrupted and electrolytes should be monitored and corrected, if necessary. Particularly, prolongation of more than 500 msec QTc or more than 60 msec from pre-treatment is associated with a higher risk of transition to TdP. If TdP occurs, tachycardia pacing by temporary pacemaker and/or infusing Mg preparations are required. After improvement in TdP, it is preferable to resume with a smaller dose of chemotherapy if there is no alternative therapy.

Atrial fibrillation

Several reports have revealed a link between cancer and atrial fibrillation (*73*). Atrial fibrillation is common in cancer patients. The development of postoperative atrial fibrillation due to surgical invasion is well documented, especially after lung cancer surgery, with rates as high as 5-28% (*74*). One epidemiological study reported that the incidence of atrial fibrillation was 2.4% at the time of cancer diagnosis, but 1.8% of patients developed new atrial fibrillation after cancer diagnosis (*75*).

Conversely, in a study of women only, 147 of 1,467 (10%) newly diagnosed atrial fibrillation patients were subsequently diagnosed with cancer (*76*). In a comparison of cancer incidence with patients without atrial fibrillation, a significantly higher hazard ratio of 1.48 was reported in patients with atrial fibrillation, even after adjusting for many confounding factors such as age, race, body mass index, hypertension, diabetes, and dyslipidemia. The results were consistent across the different types of atrial fibrillation. The cancer with the highest risk was colorectal cancer, which was reported to have about twice the risk (*76*).

When atrial fibrillation occurs, bleeding complications from thromboembolism and the anticoagulation used to treat it are also problematic, as is heart failure caused by atrial fibrillation. Although there is still no definitive statement on anticoagulation for cancer patients with atrial fibrillation, bleeding risk assessment by HAS-BLED (*77*) and thromboembolic risk by the CHA_2DS_2-VASC might be useful (*74*).

Cancer-associated thrombosis

Although cancer-related deaths are the leading cause of death in cancer patients, thromboembolism is the second most common cause, along with infections (*78*). Cancer-related, particularly venous thromboembolism is called cancer-associated thrombosis (CAT). The risk is also 4.3 times higher in cancer patients than in non-cancer patients (*79*). Causes of thromboembolism in cancer patients include cancer-related factors, patient-related factors, and treatment-related factors (*4*).

Risk Factors

Cancer-related factors include the origin of the cancer. Stomach and pancreatic cancers are at particularly high risk, followed by lung, hematologic, gynecologic, brain, kidney, and bladder cancers. Also, the higher the grade and the more advanced the stage, the higher the risk. It also often occurs within 3 months of cancer diagnosis (*79*). Patient-related factors include hereditary thrombogenic predisposition; comorbidities such as heart, lung, and renal diseases; history of venous thromboembolism; varicose veins in the legs; older age; women; and decreased activity. Treatment-related factors include surgery, radiation therapy, blood transfusion, central venous catheter placement, rest and hospitalization. Hormonal agents such as platinum, anticancer agents, L-asparaginase, and estrogen have long been known to increase the risk of disease (*80*). The occurrence of venous thromboembolism (VTE) has also been reported in more than 10% of myeloma cases (*81*), and the risk is further increased with the use of immunomodulatory drugs (IMiDs) such as lenalidomide, which play a central role in the therapy. VEGF inhibitors such as bevacizumab (*65*) and multi-kinase drugs such as sunitinib are also known to be a risk.

The Khorana score is often used in the identification of high-risk patients with CAT. A score of approximately 2.5 months shows short term thrombosis risk, and a score of 3 or more recommends appropriate VTE prophylaxis (*82*). The COMPASS-CAT RAM has also been used as a predictive score for VTE in patients with breast, colorectal, lung, and ovarian cancer during outpatient chemotherapy (*83*). Anti-hormonal therapy and the use of anthracyclines have been identified as risk factors.

Diagnosis and treatment

Pre-test probability is important in the diagnosis of VTE. Deep venous thrombosis (DVT) is typically accompanied by swelling of the affected lower limb, but is often asymptomatic. Pulmonary thromboembolism (PTE) is a cause of dyspnea and chest pain, but may be detected incidentally on enhanced computed tomography (CT) scan. Wells scores (*84*) are used to assess pre-test probability. D-dimer which is highly sensitive for VTE, can be used to deny VTE if the pretest probability is low and D-dimer is normal (*85*). If the pre-test probability is high, an imaging study should be performed. The first choice for testing for DVT is lower extremity venous ultrasound. Contrast-enhanced CT is necessary because of the ultrasound difficulty in diagnosing abdominal and pelvic areas if a proximal thrombus or PTE is suspected.

Treatment is similar for the treatment of VTE in non-cancer patients; hospitalization and outpatient care are considered depending on the severity of PTE and

DVT. The pulmonary embolism severity index (PESI) score (*86*) and vital signs are evaluated. Treatment is primarily anticoagulant; the ASCO guidelines (*87*) recommend the use of low-molecular-weight heparin (LMWH), unfractionated heparin (UFH), fondaparinux, or rivaroxaban as initial therapy. Vitamin-K antagonists are of limited use when LMWH or direct oral anti-coagulations (DOACs) are not available. The duration of use should be determined taking into account the risk of bleeding and thrombosis. Randomized control trials of DOACs for CAT compared with LMWH were shown noninferior in terms of recurrent thrombosis but more frequent bleeding (*88-90*) (Table 3). For apixaban, there was no significant difference in bleeding between the two groups (*90*). There is no clear evidence for the use of DOACs for prophylaxis; data on primary prevention in patients with high Khorana scores show that the use of DOACs prevents thrombosis but clearly increases bleeding (*91*). It is important to use them with caution in those at very high risk for thrombosis.

Late cardiotoxicity of radiotherapy

Radiation therapy is one of the mainstays of cancer treatment. Irradiation causes damage to DNA strands through the production of reactive oxygen species, which exerts an anti-tumor effect. If the irradiated area includes the heart, various cardiac injuries may occur (*92*).

Coronary artery disease

Radiation-induced coronary artery disease (RI-CAD) is a late complication of radiation therapy, occurring in many cases more than 10 years later (*93*). A recent study found that the incidence of major adverse cardiovascular events increases in proportion to the radiation dose to the heart (*94*). However, there is no threshold, and we know that there is a stochastic effect. It has long been used in Hodgkin's disease and breast cancer, but in recent years it has also been used as preoperative adjuvant or curative chemoradiation in esophageal cancer and non-small cell lung cancer, and in many cases cardiac irradiation is unavoidable. The most common sites of lesions are near the ostium of 3 coronary arteries and the left main trunk. The incidence of RI-CAD has been found to increase with the presence of classical risk factors for atherosclerotic diseases (94). Therefore, intervention according to risk factors and regular checkups are considered necessary. There are still no appropriate recommendations for invasive tests such as coronary CT.

Valvular disease

Radiation has been reported to cause damage to the valve leaflets themselves, resulting in fibrous thickening, shortening, and calcification of the valves and surrounding tissue, leading to valvular heart disease. It is common in aortic and mitral valves. The incidence of valvular heart disease after radiation therapy has been reported variously, but the incidence is less than 10%, and the incidence increases when the radiation dose to the chest exceeds 30 Gy (*4*). Echocardiography is not required routinely before or during radiation therapy, but screening at 10 years and every 5 years thereafter is recommended for high-risk patients, even if they are asymptomatic (*95*).

Pericardial disease

The incidence of acute pericarditis decreases with decreasing radiation dose. Chronic pericarditis or

Table 3. The summary of clinical trials using DOAC for cacer-associated thrombosis

	Rivaroxaban	Apixaban	Edoxaban
Study name	SELECT-D	Caravaggio	Hokusai VTE Cancer
Authors, Year (*Ref.*)	Young AM, *et al.* 2018 (*89*)	Agnelli G, *et al.* 2020 (*90*)	Raskob GE, *et al.* 2018 (*88*)
n	203 *vs.* 203	576 *vs.* 579	522 *vs.* 524
Control	Dalteparin	Dalteparin	Dalteparin
Dose	30 mg bid for the first 3 weeks followed by 20 mg once daily for a total of 6 months	10 mg twice daily for the first 7 days and 5 mg twice daily thereafter	60 mg once daily
Follow-up period	6 months	180 days	360 days
Efficacy outcome	VTE recurrence	VTE recurrence	VTE recurrence
Result, *n* (%)	8 (4) *vs.* 18 (9)	32 (5.6) *vs.* 46 (7.9)	41 (7.9) *vs.* 59 (11.3)
HR [95% CI]	0.43 [0.19 to 0.99]	0.63 [0.37 to 1.07]	0.71 [0.48 to 1.06]
p-value	-	< 0.001 for non-inferiority, 0.09 for superiority	0.09
Safety outcome	Major bleeding and clinically relevant non-major bleeding	Major bleeding according to the criteria of ISTH[†]	Major bleeding according to the criteria of ISTH[†]
Result, *n* (%)	11 (5) *vs.* 6 (3)	22 (3.8) *vs.* 23 (4.0)	36 (6.9) *vs.* 21 (4.0)
HR [95% CI]	1.83 [0.68 to 4.96]	0.82 [0.40 to 1.69]	1.77 [1.03 to 3.04]
p-value	-	0.6	0.04

[†]defined as overt bleeding that was associated with a decrease in the hemoglobin level of 2 g per deciliter or more, led to a transfusion of 2 or more units of blood, occurred in a critical site, or contributed to death. CI: Confidence Interval, ISTH: Internationl Society on Thrombosis and Haemostasis, HR: Hazard Ratio, VTE: Venous Thromboembolism,

exudative constrictive pericarditis with effusion may be seen. In rare cases, tamponade may occur, requiring pericardiocentesis. In the case of drug-refractory constrictive pericarditis, pericardiectomy might be required (*4*).

Current Remaining Issues and Future Developments

Onco-cardiology is a field that has just begun to dawn, but is gradually being recognized as a new problem for cancer survivors. Genome analysis is being conducted for chemotherapy-related cardiomyopathy. Mutations in the Titin gene, one of the causative genes of idiopathic dilated cardiomyopathy and other diseases, have been reported to be involved in the development of chemotherapy-related cardiomyopathy (*96,97*). Analysis of such gene mutations or SNPs may allow identification of high-risk groups for chemotherapy-related cardiomyopathy before treatment.

Another issue is the transitional care of the increasing number of childhood cancer survivors. Childhood cancer survivors have a significantly increased incidence of heart failure and other cardiac problems above the age of 35 when compared to their non-cancer survivor siblings and this occurs as a problem in later life (*98*). How to conduct regular follow-up after adulthood has become an issue. There is also an increase in the number of cancers in the younger age group, known as the adolescence and young adult (AYA) generation. When cardiotoxicity occurs at a young age, it is necessary to create a system to provide social, economic, and emotional support in collaboration with various specialties.

Conclusion

The prognosis of cancer has improved by establishment of cancer therapy and development of novel chemotherapy. Now that the patients can live longer, the physician needs to pay attention for cardiotoxicity of cancer therapy and complications of cardiovascular disease. GLS and some biomarkers such as BNP and troponin are useful for early detection of cardiotoxicity, and we have to check them when a high risk drug is used for patients who have high risk background. Dexrazoxane, beta-blockers, ACE inhibitors, and statins are possible treatments to prevent cardiotoxicity. ICI-associated myocarditis is rare but has poor prognosis when it occurs. CMR may be able to detect problems in the early phase and early initiation of high-dose steroids has been reported to improve prognosis. Ischemic heart disease, hypertension and arrhythmia occur due to aging of cancer survivors and using some drugs. CAT can occur, especially in patients with pancreatic and stomach cancer. Randomized control trials of DOACs for CAT compared with LMWH were shown noninferior in terms of recurrent thrombosis but more frequent bleeding except for apixaban. Radiotherapy damage can occur to the coronary artery, as well as valvular and pericardial disease in the late phase.

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A newly established monoclonal antibody against ERCC1 detects major isoforms of ERCC1 in gastric cancer

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Abstract: Identifying patients resistant to cisplatin treatment is expected to improve cisplatin-based chemotherapy for various types of cancers. Excision repair cross-complementing group 1 (ERCC1) is involved in several repair processes of cisplatin-induced DNA crosslinks. ERCC1 overexpression is reported as a candidate prognostic factor and considered to cause cisplatin resistance in major solid cancers. However, anti-ERCC1 antibodies capable of evaluating expression levels of ERCC1 in clinical specimens were not fully optimized. A mouse monoclonal antibody against human ERCC1 was generated in this study. The developed antibody 9D11 specifically detected isoforms of 201, 202, 203 but not 204, which lacks the exon 3 coding region. To evaluate the diagnostic usefulness of this antibody, we have focused on gastric cancer because it is one of the major cancers in Japan. When ERCC1 expression was analyzed in seventeen kinds of human gastric cancer cell lines, all the cell lines were found to express either 201, 202, and/or 203 as major isoforms of ERCC1, but not 204 by Western blotting analysis. Immunohistochemical staining showed that ERCC1 protein was exclusively detected in nuclei of the cells and a moderate level of constant positivity was observed in nuclei of vascular endothelial cells. It showed a clear staining pattern in clinical specimens of gastric cancers. Antibody 9D11 may thus be useful for estimating expression levels of ERCC1 in clinical specimens.

***Keywords*:** excision repair cross-complementing group 1, gastric tumor, cisplatin, predictive biomarker

Introduction

Platinum compounds such as cisplatin (CDDP), carboplatin and oxaliplatin have been used as the standard of care for various cancer patients including gastric, non-small cell lung, head and neck, and cervical cancers (*1-5*). These anti-cancer agents induce cell death accompanying inhibition of DNA repair pathways. CDDP forms intra-strand DNA adducts and inter-strand crosslinks (ICL), which inhibit DNA repair and replication to induce cell death (*6*). DNA lesions caused by CDDP are mainly repaired by nucleotide excision repair (NER) and Fanconi anemia pathway. NER is the major pathway for elimination of DNA adducts generated by CDDP (*7*). In NER pathway, excision repair cross-complementing group 1 (ERCC1) forms a tight complex with xeroderma pigmentosum group F (XPF) to function as a DNA endonuclease (*7*). ERCC1-XPF heterodimer recognizes platinum-DNA adducts and is required for introducing incision at the ICL lesions (*8,9*). For this reason, ERCC1 plays an important role in repair of DNA damage introduced by platinum compounds. It

has been suggested that ERCC1 overexpression may lead to chemoresistance to platinum-based therapy in cancer cells.

It has been reported that ERCC1 overexpression was associated with poor prognosis after CDDP treatment of patients such as gastric and non-small cell lung cancers (*1,10-13*). These reports suggested that high ERCC1 expression levels were correlated with resistance to platinum agents such as CDDP. In advanced gastric cancer, the measurement of *ERCC1* mRNA level showed a positive association with resistance to cisplatin and 5-fluorouracil-based therapy (*1*). Thus, it is considered that ERCC1 overexpression level may become a predictive biomarker for CDDP treatment. Low expression levels of ERCC1 were also reported to increase sensitivity of lung cancer cells to PARP inhibitors (*14,15*).

The ERCC1 gene produces four isoforms (201, 202, 203 and 204) by alternative splicing. Friboulet *et al.* reported that only ERCC1 isoform 202 contributed to develop cisplatin resistance in a xenograft model of lung cancer cells A549 (*16,17*). However, each isoform contains particular domains for different functions and it is not elucidated whether other isoforms function or not in certain cancer types for the repair of CDDP and other platinum agents. Considering this situation, the antibody that detects not only 202 but also other major isoforms of ERCC1 may be useful for evaluation of sensitivity to cisplatin and other platinum compounds.

Recently, several anti-ERCC1 antibodies for immunohistochemical staining of clinical specimen were developed (*16,18-21*). Anti-ERCC1 monoclonal antibody 8F1 was available for evaluating the ERCC1 levels in tumor samples of cancer patients. However, 8F1 did not specifically detect ERCC1 and was found to be cross-reactive with an unrelated protein (*16,18*).

In this study, to evaluate the expression level of ERCC1 in tumor samples by immunohistochemical staining, we developed a novel anti-ERCC1 monoclonal antibody 9D11 using a method to obtain a high affinity antibody. We showed that 9D11 antibody can recognize ERCC1s three major isoforms containing exon 3 coding regions and detected ERCC1 protein in all seventeen gastric cancer cell lines. It was further shown to detect ERCC1 in immunohistochemical staining of tumor specimens from patients.

Materials and Methods

Cell culture and reagents

Hybridoma cells were cultured in Hybridoma-SFM (Gibco, NY, USA) and gastric cancer cell lines MKN45, HSC-39, HSC-40A, HSC-43, HSC-44PE, HSC-45, HSC-57, HSC-58, HSC-59, HSC-60, HSC-64, SH101-P4, MKN-1, MKN-28, MKN-74 and KATO-III were cultured in RPMI1640 medium (Gibco) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin (Invitrogen, MA, USA) (*22,23*). Gastric cancer cell line GCIY was cultured in MEM medium (Gibco) supplemented with 15% fetal bovine serum (Gibco) and 1% penicillin-streptomycin (Invitrogen). HeLa cells were cultured with MEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were maintained in a humidified atmosphere with 5% CO_2 at 37°C.

E. coli strains and plasmids

Plasmid pET-ERCC1 encodes human ERCC1 isoform 202 downstream of P_{T7} promoter of pET-Duet1. This plasmid contains an N-terminal His6 tag conjugated with ERCC1. For construction of expression plasmids of other ERCC1 isoforms in *E. coli*, the pET-ERCC1 was used as a template. The pET-E201, pET-E203 and pET-E204 all contain the N-terminal His6-tag conjugated human ERCC1 isoforms 201, 203 and 204 cassettes, respectively. These genes were cloned into the *EcoR* I-*Sal* I double digested pET-Duet1 (GenScript, Tokyo, Japan). *E. coli* BL21 (DE3) was utilized to induce expression of ERCC1 isoforms. For expression in human cells, plasmid RC208787 and RC228204 were purchased from ORIGENE to express the ERCC1 isoforms 201 and 203 with C-terminal Myc and FLAG tags, in HeLa cells. For expression of ERCC1 isoform 204 in HeLa cells, plasmid pCDNA-E204 was constructed from pCDNA3-HsERCC1, which contains isoform 202 cDNA. This plasmid encodes human ERCC1 isoform 204 without tags downstream of the cytomegalovirus promoter.

Purification of recombinant human ERCC1 isoform 202 as the antigen

E. coli transformed with plasmid pET-ERCC1 was grown in Luria-Bertani broth containing 100 μg/mL ampicillin at 37°C until OD_{600} reached approximately 0.5. Isopropyl-β-$_D$-1-thiogalactopyranoside (IPTG) at 1 mM was then added to induce expression of ERCC1 and cultured further at 37°C for 5 hr. Cells were centrifuged at 2500 xg for 20 min, washed with PBS (-) harvested by centrifugation and sonicated into buffer A (0.5 M NaCl, 5% (v/v) glycerol, 50 mM Tris-HCl (pH8.0), 1 mM PMSF). After centrifugation at 1700 xg for 25 min, supernatant (soluble fraction) was mixed into 5 ml of TALON Metal Affinity Resin (Clontech, CA, USA) and incubated on ice for 30 min, loaded onto the column and washed with 10 column volumes of buffer A containing 5 mM imidazole. His-tagged proteins were eluted with 5 column volumes of buffer A containing 500 mM imidazole, and pooled using measurement of A_{280}. This fraction was passed through Amicon Ultra (30K MWCO) (Millipore, MA, USA) to exchange the buffer and concentrate recombinant proteins. The purity

of the eluted fraction was confirmed with SDS-PAGE and Western blotting using anti-ERCC1 antibody 8F1 (Abcam, Cambridge, UK).

Immunization and screening of ERCC1 monoclonal antibodies

Recombinant ERCC1 (ProSpec-Tany TechnoGene Ltd., Rehovot, Israel) of isoform 202 harboring N-terminal His6 tag was used to immunize three GANP mice to generate monoclonal antibodies and purified recombinant ERCC1 isoform 202 as described above were used for evaluation of conditioned medium in hybridoma clones by ELISA (TransGenic Inc., Fukuoka, Japan). Briefly, NUNC Maxi Sorp F96 plates (Thermo Fisher Scientific, MA, USA) were coated with 1 μg / ml recombinant human ERCC1 isoform 202 for 1 hr at room temperature and incubated with PBS-T containing 0.5 % gelatin overnight at 4 °C. Then, conditioned medium from hybridoma culture supernatant was added and incubated for 1 hr at room temperature. Substrate solution containing *o*-phenylenediamine (OPD) was added to wells and the reaction was stopped with 1 N H_2SO_4. Plates were analyzed using a plate reader at an absorbance of 490 nm.

Western blot analysis

Western blotting was performed as described previously (*24*). Cell extracts were prepared with Laemmli's buffer and separated by SDS-polyacrylamide gel electrophoresis and transferred onto PVDF membranes. The following antibodies were used for Western blotting: anti-ERCC1 (8F1, Abcam), anti-ERCC1 (FL297) (Santa Cruz Biotechnology, CA, USA) and anti-β-actin (Sigma-Aldrich, MO, USA). Immune complexes were visualized using a horseradish peroxidase-linked secondary antibody and enhanced chemiluminescence (Millipore, MA, USA). Image quantification was performed with Image J software (NIH).

Quantitative RT-PCR (qRT-PCR)

RNA prepared was reverse transcribed using a High Capacity Reverse Transcription Kit (Thermo Fisher Scientific, MA, USA). The quantitative RT-PCR (qRT-PCR) analysis was performed using SYBR Green with the CFX96 Real-Time System (Bio-Rad, CA, USA) and StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). The mRNA levels were normalized by *GUSB*. The primers for qRT-PCR were synthesized by Eurofins Genomics: For *ERCC1*, forward primer (F2) 5'-GGGAATTTGGCGACGTAATTC-3', and reverse primer (R2) 5'- GCGGAGGCTGAGGAACAG-3' and for *GUSB*, forward primer 5'-GCCTGCGTCCCACCTAGAAT-3' and reverse primer 5'-ACATACGGAGCCCCCTTGTC-3' were used, respectively.

Transfection

MKN45 (2.3×10^5 cells) and HeLa (1.0×10^5 cells) were seeded onto 6-well and 12-well plates, respectively. Transfection with plasmid DNA (pCDNA3 Hs-ERCC1) was performed using Lipofectamine 3000 (Life Technologies, CA, USA).

For siRNA transfection to HeLa cells, Lipofectamine RNAiMAX (Life Technologies) was used. Individual siRNAs were used at a final concentration of 10 nM in Opti-MEM. The siRNAs for ERCC1 (5'-GGCCAAGCC CUUAUUCCGAUCUACA-3', 5'-UGUAGAUCGGAA UAAGGGCUUGGCCAC-3', (hs.Ri.ERCC1.13.1) were purchased from Integrated DNA Technologies. DS NC1 siRNA (51-01-14-04) (Integrated DNA Technologies, IA, USA) was used as a negative control (siN.C.).

Construction of a stable cell line expressing human ERCC1 isoform 202

MKN45 cells (2.3×10^5 cells) were seeded onto 6-well plates and transfected with plasmid pCDNA3 Hs-ERCC1, that was digested with restriction enzyme *Pvu* I and purified with Gene Jet Kit (Thermo Fisher Scientific). In the N- or C-terminal region, tag was not conjugated. Two days after transfection, cells were started in culture with medium containing 800 μg/mL of G418 for 19 days. After treatment of G418, surviving colonies were isolated and cultured further for 4 days. Stable cell lines expressing human ERCC1 were selected by qRT-PCR with SuperPrep Cell lysis and RT kit for qPCR (TOYOBO, Osaka, Japan) and confirmed by Western blotting with anti-ERCC1 (4F9 and FL297).

Preparation of tumor xenografts derived from stable cell lines overexpressing human ERCC1 isoform 202 in mice

MKN45 cells or stable cell lines expressing human ERCC1 isoform 202 (1×10^6 cells) were mixed with Growth Factor Reduced Matrigel (BD Biosciences, NJ, USA) and injected subcutaneously into flanks of 5-week-old Balb/c-*nu*/*nu* nude mice (Japan SLC, Inc., Shizuoka, Japan). Tumor diameters were measured with micrometer calipers, and tumor volumes were calculated using the following formula: (largest diameter) × (smallest diameter)^2/2. Animal studies were approved by the Animal Experimental Committee of the National Cancer Center and performed following the Guidelines for Animal Experiments of the National Cancer Center. When tumor volumes reached approximately 100-220 mm^3, mice were sacrificed and tumors were fixed by formalin. Then, paraffin-embedded sections were prepared.

Immunohistochemical staining

For immunohistochemical staining of paraffin-

embedded tissue and tumor sections, the sections were deparaffinized in xylene and rehydrated. Antigen retrieval was performed with Envision FLEX target retrieval solution (Dako Japan Inc., Kyoto, Japan), Low or High pH (Dako Japan Inc) by autoclave treatment at 121°C for 10 min. After blocking of endogenous peroxidase activity with EnVision FLEX Peroxidase Blocking Reagent (Dako Japan Inc.) for 5-10 min, samples were washed with Wash buffer (Dako Japan Inc.) and incubated with anti-human ERCC1 antibody diluted with Antibody Diluent (Dako Japan Inc.) for 20 min at room temperature. After washing the sections, samples were incubated with Polymer Reagent (Dako Japan Inc.) for 20 min at room temperature. After washing the sections, antibody complexes on the slides were detected with 3,3'-diaminobenzidine substrate (Dako Japan Inc.). Immunohistochemical analyses were also carried out with Ventana Bench Mark XT Automated Stainer (Ventana Medical Systems Inc., Tucson, AZ, USA) using OptiView DAB Universal Kit with the procedure of OptiView DAB IHC v4 following standard protocols.

Study samples

Histologically proven gastric cancer patient specimens used for this study were approved by the Institutional Ethics Committees of Nagasaki University and National Cancer Center, within which the work was undertaken and it conforms to the provisions of the Declaration of Helsinki.

Purification of anti-ERCC1 monoclonal antibody (clone No. 9D11)

Fifty ml of Protein G-Sepharose (GE Healthcare Japan, Tokyo, Japan) was mixed with 2,500 ml of conditioned medium. After washing the column with PBS (-), the antibody was eluted with glycine buffer (pH 3.0) and collected into new tubes (6 ml/fraction). After neutralization, 280 nm absorbance was measured and the fractions containing antibodies were pooled and dialyzed with PBS (-) and concentrated with ultrafilters.

Expression of ERCC1 isoforms in E. coli and preparation of whole cell lysates

Plasmids containing an expression cassette of human ERCC1 isoform 201, 202, 203 and 204, respectively, were transformed into *E. coli* BL21 (DE3) and cultured in LB medium containing 100 μg/ml ampicillin until OD_{600} reached 0.4-0.5. Then, after treatment with 1 mM IPTG approximately for 5 hr, cells were harvested by centrifugation at 5700 *xg* for 10 min. *E. coli* expressing ERCC1 were lysed into Laemmli's buffer containing Complete Protease Inhibitor Cocktail (Roche, Basel, Swiss) and the resulting supernatant was obtained by centrifugation at 13,000 rpm for 10 min (MA-2024 rotor, KUBOTA, Tokyo, Japan) and used as whole cell lysates.

Immunofluorescence staining

HeLa cells were seeded and transfected with siRNA of negative control (siN.C.) or *ERCC1* in chamber slides and cultured at 37°C for 3 days. Cells were fixed with methanol and treated with PBS(-)-1% BSA-10% FBS for 2 hr. The following antibodies were used for immunofluorescence staining: anti-ERCC1 (9D11). Immune complexes were detected using AlexaFluor 594-conjugated mouse secondary antibodies (A-11005) (Molecular Probes, OR, USA). Finally, cells were counterstained with 4',6-diamidino-2-phenylindole (DAPI) using VECTASHIELD Mounting Medium (H-1200) (VECTOR Laboratories, CA, USA).

Results

Screening of novel monoclonal antibodies against human ERCC1

To evaluate the expression level of ERCC1 in human tumor tissues, we developed novel monoclonal antibodies targeting human ERCC1 that are useful for immunohistochemical staining. To establish monoclonal antibodies to ERCC1, GANP mice (*25*), which can develop high affinity antibodies, were immunized with the purified recombinant ERCC1 isoform 202 (Figure S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=23*). Thirty clones of hybridoma cells were obtained and the conditioned media, which can recognize human ERCC1, were screened. Analysis with ELISA showed that all of the conditioned media could bind to the purified ERCC1 (Figure S2, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=23*). To evaluate antigen specificities of antibodies in conditioned media, we also performed Western blot analysis using cell lysate of gastric cancer cells MKN45, which transiently overexpressed human ERCC1 isoform 202. Twenty-six clones of conditioned media showed detection of ERCC1 at a molecular weight of 37 kDa (data not shown).

Next, we assessed reactivity of antibodies against ERCC1 in immunohistochemistry. For this purpose, to generate xenograft models of human ERCC1 overexpressing and parental tumors, we established stable cell lines of human stomach cancer MKN45 cells that overexpress ERCC1. Clone 14, 2, and 21 expressed 3.6, 2.5, and 1.2-fold levels of ERCC1, respectively, compared with parental MKN45 cells in Western blots using commercially available antibody 8F1 against ERCC1 (Figure 1A). These MKN45 clones and parental cells were subcutaneously transplanted into nude mice to obtain tumor xenografts. Paraffin-embedded tumor

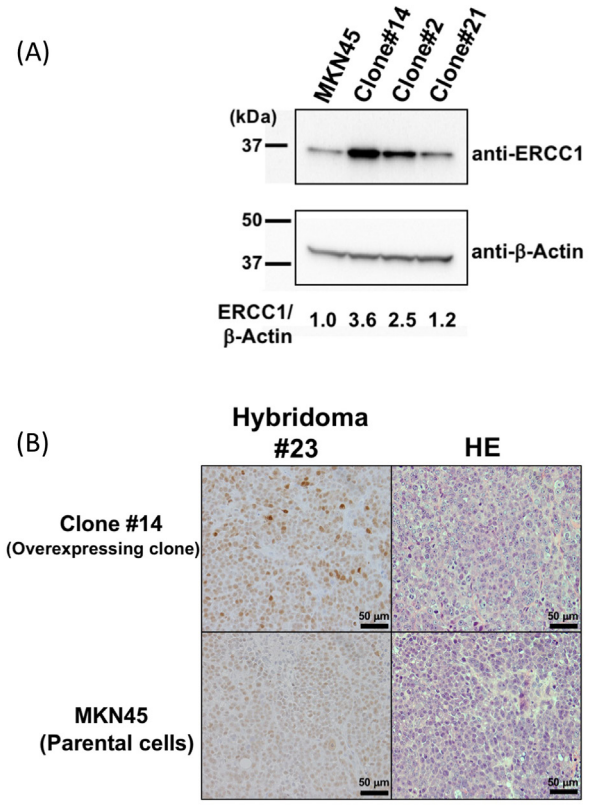

Figure 1. Establishment and evaluation of ERCC1 monoclonal antibody 9D11. (A) Construction of MKN45 stable cell lines expressing human ERCC1 isoform 202. Expression of ERCC1 in stable cell lines was verified by Western blotting with antibody 4F9. The expression level of ERCC1 was 3.6-fold higher in MKN45 clone #14 as compared with the parent cell line MKN45. **(B)** Evaluation of conditioned media of the hybridoma clone #23 that produces antibody 9D11 by immunohistochemical staining. Expression of ERCC1 in the xenograft derived from MKN45 clone #14 (the upper panels) that overexpresses ERCC1 showed a higher positivity compared with the xenograft derived from parental MKN45 cells (the lower panels). Bar, 50 μm.

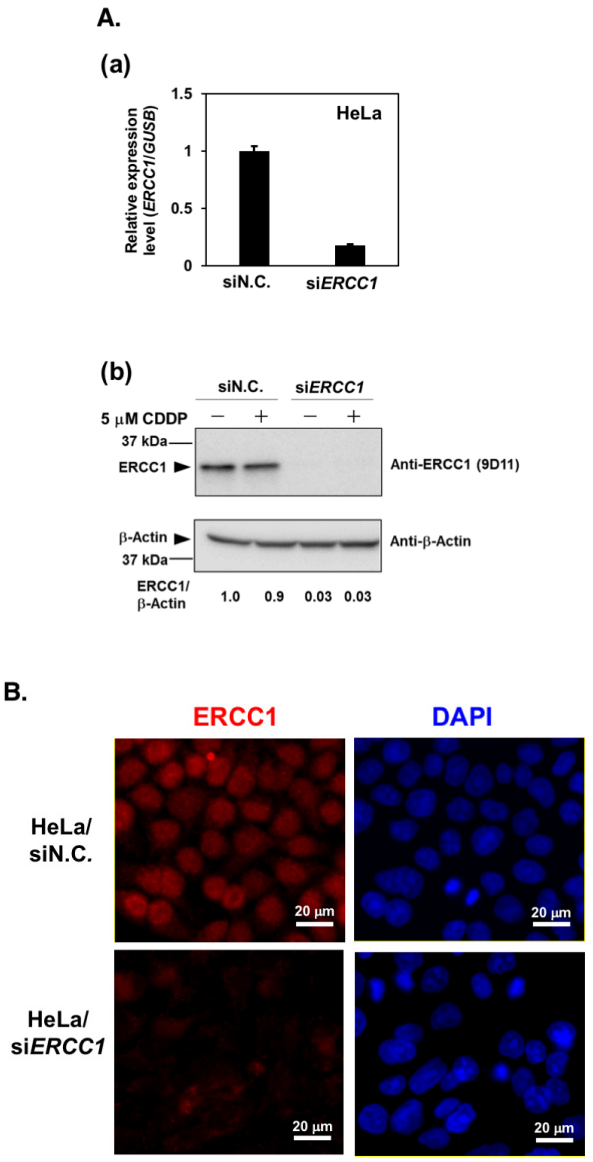

Figure 2. Immunofluorescent staining with antibody 9D11 in ERCC1 knock-down HeLa cells. (A) (a) Real-time PCR analysis of ERCC1 mRNA in knock-down cells three days after transfection of siRNAs, ERCC1 or siN.C. into HeLa cells. The siRNA treated HeLa showed a decreased *ERCC1* mRNA compared to siN.C. condition. (b) Western blot analysis of ERCC1 protein level with antibody 9D11 in knock-down cells and control cells (siN.C.) after treatment with 5 μM CDDP or in the absence of CDDP for 10 hr two days after transfection. **(B)** ERCC1 was detected by immunofluorescent staining with antibody 9D11 (red color) with DAPI staining (blue color) in HeLa cells three days after transfection of si*ERCC1* or control (siN.C.). Bars, 20 μm.

sections were then prepared from ERCC1-overexpressed tumors. We next performed immunohistochemical staining to validate twenty-six clones using hybridoma conditioned media. It revealed that hybridoma conditioned media of six clones 3, 12, 17, 23, 24, and 28 more intensely stained ERCC1 in the nuclei of the tumors derived from MKN45 clone 14 that overexpresses ERCC1 as compared with those derived from parental MKN45. Especially, hybridoma conditioned media of clone 23 (antibody was named 9D11) showed a most clear nuclear staining pattern in ERCC1-overexpressed tumor sections (Figure 1B). Accordingly, we focused on 9D11 as a novel anti-ERCC1 antibody and purified the antibody from conditioned medium. ELISA analysis showed that 9D11 antibody could recognize recombinant ERCC1 isoform 202, in a concentration-dependent manner (data not shown).

Characterization of anti-ERCC1 monoclonal antibody 9D11

To validate whether antibody 9D11 specifically recognizes ERCC1, *ERCC1* was knocked down with siRNA in HeLa cells. Real-time PCR using F2-R2 primers for exon 4-5, which can amplify all isoforms and Western blots supported the reduced level of ERCC1 (Figure 2A a & b). We noted that HeLa cells express isoform 202 and/or 203 but not 201 and 204.

Immunofluorescence staining showed that 9D11 antibody exhibited positive staining of HeLa cell nuclei,

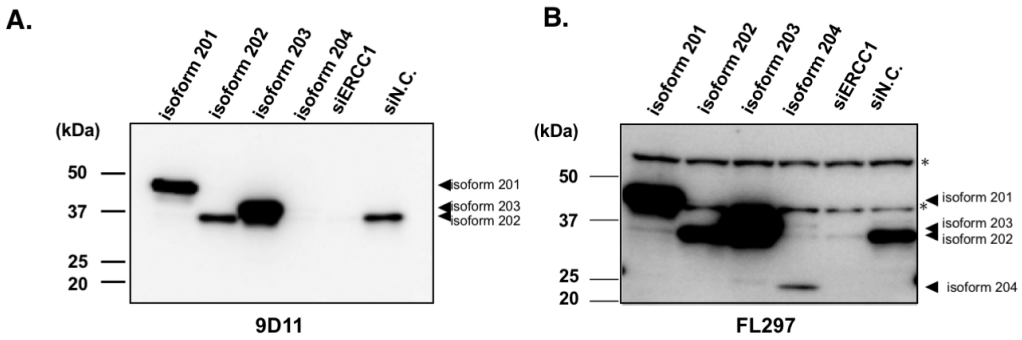

Figure 3. 9D11 antibody recognizes ERCC1 isoforms 201, 202, and 203 but not 204. Isoforms 201, 202, 203 and 204 were transiently expressed in *ERCC1* knock-down HeLa cells and whole cell extracts were subjected to Western blot analysis using anti-ERCC1 antibodies, 9D11 **(A)**, and FL297 **(B)**. *, non-specific band.

whereas almost no staining was observed in the knocked down cells with *ERCC1* siRNA (Figure 2B). The results suggested that 9D11 antibody specifically recognizes ERCC1 protein.

9D11 antibody recognizes isoforms 201, 202, and 203 but not 204

To clarify which isoforms are recognized by 9D11 antibody, we transiently transfected isoforms 201, 202, 203, and 204 expression vectors into ERCC1 knocked-down HeLa cells, respectively. As shown in Figure 3A, antibody 9D11 could detect isoforms 201, 202, and 203 but not isoform 204 and no other unrelated proteins, whereas polyclonal antibody FL297 detected all isoforms but non-specific bands were also detected (asterisks in Figure 3B).

9D11 antibody detects ERCC1 in seventeen gastric cell lines

To examine whether 9D11 detects major isoforms of ERCC1 in gastric cancer, we next examined whether 9D11 antibody can detect ERCC1 protein in seventeen gastric cancer cell lines of various types. When we measured *ERCC1* mRNA expression levels by real-time PCR using F2-R2 primer set that detects all four isoforms of ERCC1, all cell lines showed expression of ERCC1 at various levels (Figure 4A). We next carried out Western blot analysis of ERCC1 using 9D11. As shown in Figure 4B, all seventeen cell lines expressed ERCC1 protein at 37 kDa that matches the size of isoforms 202 and 203. In these gastric cancer cell lines, the presence of isoform 204 at 20-25 kDa was not detected with a commercially available polyclonal antibody FL297 that can recognize all four isoforms (Figure 4C). The bands (marked with asterisks) at the upper position of the 202/203 isoform band detected by FL297 were considered to be cross-reactive non-specific bands, because in HeLa cells, knockdown with siRNA did not affect the expression of bands at similar sizes (Figure 3B). These Western blot results with 9D11 and FL297 antibodies suggested that 202 and/or 203 could be the major isoforms in gastric cancer cells. We also noted that the mRNA and protein levels of ERCC1 did not show a clear correlation. The specific detection of ERCC1 major forms by 9D11 antibody suggests that 9D11 could be useful to detect ERCC1 levels in gastric cancers.

Evaluation of staining with clinical specimens

To further evaluate the reactivity of 9D11, we performed immunohistochemical staining using the paraffin-embedded sections of gastric cancers (Figure 5A). As a result, conditioned medium of hybridoma 9D11 showed a clear pattern of nuclear staining of ERCC1 in the specimen of poorly differentiated adenocarcinoma. Ganglion cells exhibited a high staining level of ERCC1, which was consistent with a previous report using ERCC1 antibody 4F9 (*26*), while little cytoplasmic staining was observed (Figure 5A). We also observed that vascular endothelial cells show a moderate level of constant staining.

9D11 antibody was also prepared from a large scale hybridoma culture and evaluated by immunohistochemical staining with paraffin-embedded tumor sections from several gastric cancer patients. As in the case with the small scale preparation, 9D11 antibody intensely stained nuclei (Figure 5B). Specimens from different patients showed diverse levels of staining. Taken together, analysis by Western blotting, ELISA and immunohistochemical staining demonstrated that antibody 9D11 can specifically recognize human ERCC1.

Discussion

We developed a novel ERCC1 monoclonal antibody 9D11 useful for detection of three isoforms 201, 202, and 203 containing exon 3 using GANP mouse, from which high affinity antibodies can be generated because of higher rates of mutation in the variable region of antibodies (*25*). Our results using knockdown experiments with siRNA of *ERCC1* showed that

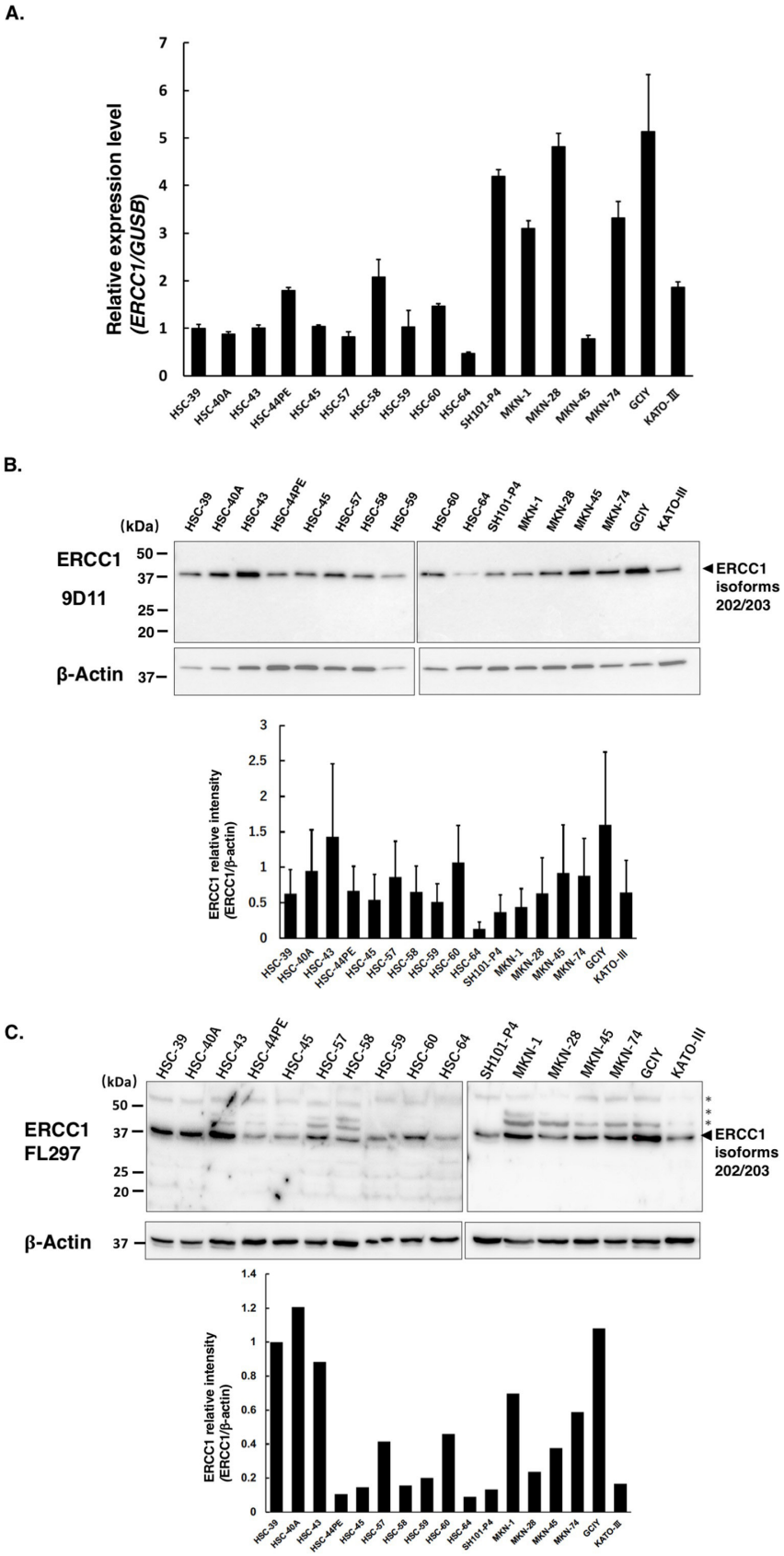

Figure 4. Analysis of *ERCC1* mRNA expression and ERCC1 protein isoforms in seventeen gastric cancer cell lines. (A) The mRNA levels of seventeen gastric cancer cell lines were analyzed by real-time PCR using F2-R2 primer set (mean + SE). **(B)** The Western blot analysis of ERCC1 using 9D11 antibody (upper panel) and the normalized ERCC1 levels by β-actin levels are shown in the lower panel (n = 2-3). **(C)** The Western blot analysis of ERCC1 detected with FL297 polyclonal antibody (upper panel) and the normalized ERCC1 levels by β-actin level are shown in the lower panel. ERCC1 isoforms 202 and/or 203 are detected at 37 kDa. The upper bands (asterisks) are cross reactive non-specific bands.

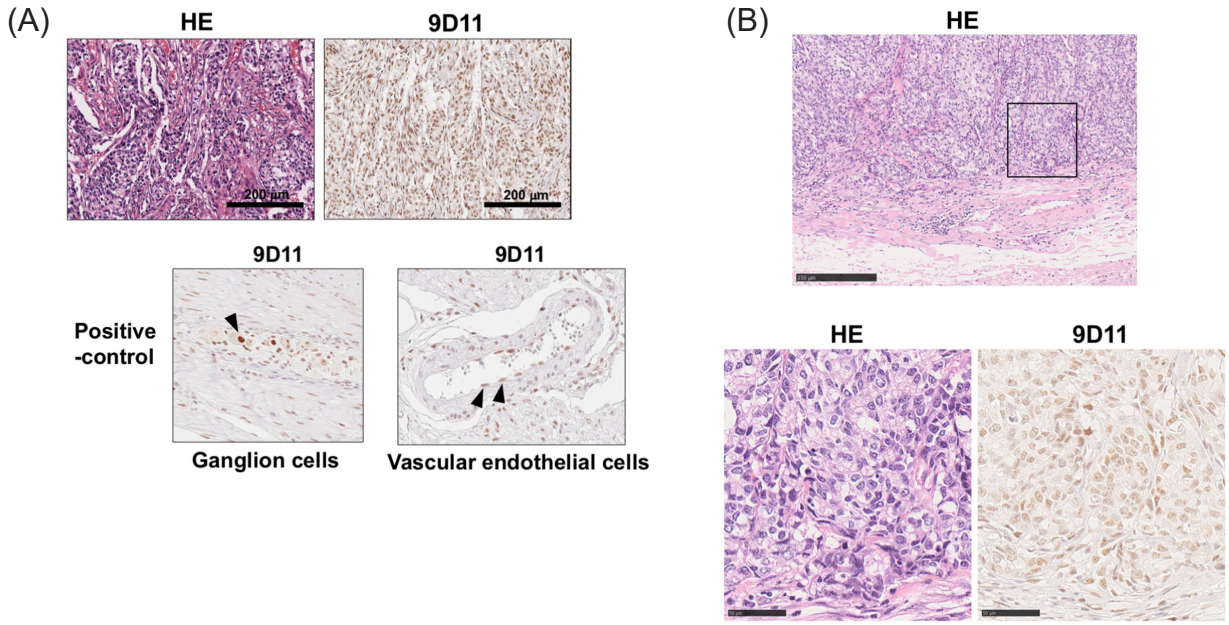

Figure 5. Immunohistochemical staining with 9D11 antibody in paraffin-embedded tumor tissue samples of gastric cancer patients. (A) A gastric cancer specimen of poorly differentiated adenocarcinoma (the top left panel, hematoxylin-eosin staining) was stained with 9D11 antibody intensely in nuclei (the top right panel) as well as ganglion cells (the bottom left panel, arrow head) and vascular endothelial cells (the bottom right panel, arrow heads). Bars, 200 μm. **(B)** 9D11 antibody was also prepared from the large scale hybridoma culture and evaluated by immunohistochemical staining with paraffin-embedded sections of poorly differentiated adenocarcinoma from gastric cancer patients. In the top panel, hematoxylin-eosin staining is shown at a lower magnification. The immunostaining of the surrounded square area is shown in the lower right panel with hematoxylin-eosin staining (the lower left panel). The bar in the top panel, 250 μm. Bars in lower panels, 50 μm.

antibody 9D11 is a specific antibody against ERCC1. We further evaluated whether this antibody can accurately detect the expression level of ERCC1 and is useful for immunohistochemical staining of tumor tissues using paraffin-embedded sections. Immunohistochemical staining using sections from gastric cancers showed an intense staining of nuclei (Figure 5A and 5B). Taken together, this study suggested that 9D11 antibody will be valuable as a novel monoclonal antibody to detect major isoforms of ERCC1 specifically in gastric cancers.

In this study, we showed that 9D11 antibody detected three isoforms 201, 202 and 203 but not isoform 204. We noted that polyclonal FL-297 antibody, which is able to recognize all four isoforms, detected only isoform 202 and/or 203 in HeLa cells and seventeen kinds of gastric cancer cell lines. Therefore, ERCC1 isoform 202 and/or 203 could be major isoforms at least in gastric cancers. This also implies that 9D11 might be useful to evaluate ERCC1 protein levels in gastric cancer specimen.

Monoclonal antibody 4F9 has been recently reported to specifically recognize isoforms 201, 202 and 203 of ERCC1 and was used to measure ERCC1 level in archival formalin-fixed paraffin-embedded colorectal cancer specimen (*26*). However, the correlation of *ERCC1* mRNA level and ERCC1 protein detection by 4F9 has not been investigated comprehensively. Using a proximity ligation assay method, Kuo *et al.* showed specific detection of functional ERCC1 isoform 202 with newly established monoclonal antibodies 2C11, 7C3 and 10D10 with 4F9 (*27*). However, each isoform contains particular sets of functional domains. Only the C-terminal domain structure of ERCC1 isoform 202 was analyzed by crystal structure analysis (*28,29*) and the structures and functions of the N-terminal domain and other isoforms have not been clarified yet. Several types of platinum agents, including carboplatin and oxaliplatin have been clinically used. Oxaliplatin is reported to kill cells by ribosome biogenesis stress (*30*), suggesting that the different processing of the lesions could be induced by other platinum agents from that induced by CDDP. There may be thus possibilities that other isoforms, such as 201 and 203 may function in DNA repair pathways after induction of lesions by other types of platinum agents. Therefore, the antibody that detects major isoforms of ERCC1, 201, 202, and 203, may be useful for evaluation of sensitivity to cisplatin and other platinum compounds.

In this study, we also showed that the established 9D11 antibody can be used to detect ERCC1 in archival formalin-fixed paraffin-embedded cancer and normal tissues. 9D11 antibody is therefore expected to be useful for measurement of ERCC1 levels in tumor specimens to predict sensitivity to chemotherapeutic agents, including CDDP and other platinum agents. Since other types of cancer and cancer cells were not evaluated in this study, our observation is limited to gastric cancers. It should therefore be examined whether this antibody can detect major isoforms of ERCC1 in other types of cancers. It

is further necessary to study the correlation between the therapeutic effect/prognosis after cisplatin-based chemotherapy and ERCC1 overexpression level in actual clinical practice for gastric and other cancers.

In the TCGA database (*31,32*), amplification of the *ERCC1* gene is found in cancers of various types not only for gastric cancers but also for endometrial carcinoma, pancreatic adenocarcinoma, cervical squamous cell carcinoma, non-small cell lung carcinoma, hepatocellular carcinoma, invasive breast carcinoma and pleural mesothelioma. Deletion of the *ERCC1* gene is found in diffuse glioma, and mature B-cell neoplasm (Figure S3, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=23*). It is therefore suggested that both overexpression and decreased levels of ERCC1 protein level could be present in cancers and these ERCC1 states may affect responses to chemotherapies including cisplatin treatment.

As previously reported, cell death is also effectively induced in ERCC1 deficient lung cancer cells with olaparib and niraparib (*14*), poly(ADP-ribose) polymerase (PARP) inhibitors. A low expression level of ERCC1 in cancer cells is reported to be associated with a high sensitivity to ionizing radiation and UV irradiation (*33*). Additionally, it was reported that cells derived from XFE progeroid syndrome patients exhibit low expression levels of ERCC1 and a high sensitivity to mitomycin C (*34*). It should be therefore further evaluated whether 9D11 antibody is useful for prediction of therapeutic efficacy including chemotherapy with CDDP, other platinum agents, mitomycin C, PARP inhibitors and radiotherapy.

Conclusions

In this study, we developed a new ERCC1 monoclonal antibody that detects all ERCC1 major isoforms, 201, 202, 203, in gastric cancer cells. We showed that this antibody can specifically detect the expression level of ERCC1 in immunohistochemical staining of paraffin-embedded tumor sections, suggesting that 9D11 monoclonal antibody will be valuable to detect major isoforms of ERCC1 in gastric cancers and possibly in various types of cancers.

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A single-center descriptive study of untraced sources of infection among new cases of coronavirus disease in Tokyo, Japan

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Abstract: We investigated possible sources of newly infected patients with coronavirus disease (COVID-19) after the fourth wave in order to explore unknown sources. Retrospective chart review on all the confirmed patients with COVID-19 admitted to the National Center for Global Health and Medicine (NCGM) in Tokyo, Japan was conducted from May 22 through June 29, 2021. Among the 22 participants, 14 (64%) had a history of known high-risk infection behaviors. Of those, 12 reported that their activities involved eating and drinking. In addition, there were 24 high-risk situations, of those, 21 (88%) were related to indoor dining, and masks were not worn in 22 situations (92%). New source of infection has not been identified. In situations with a high known risk of infection, many cases were related to eating and drinking, and insufficient use of masks was evident. Raising risk awareness on infection prevention and control of COVID-19 is urgently needed.

***Keywords*:** coronavirus disease, untraced sources of infection, high-risk infection behavior, indoor dining, mask

Introduction

Since the end of 2019, an accumulation of severe pneumonias of unknown cause was reported in Wuhan City, Hubei Province, China, which was subsequently found to be a coronavirus disease (COVID-19) (*1*). In Japan, there have been four waves of COVID-19 as of August 1, 2021 (*2*). Even if the number of new cases declines, there may be an unknown source of infection, which may serve as the origin of the next wave of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. When the number of newly infected patients in the fourth wave showed a downward trajectory, we conducted an exploratory investigation into possible sources of infection of newly infected patients after the fourth wave.

A review on confirmed patients with COVID-19 conducted after the fourth wave

We conducted a retrospective chart review on all the confirmed patients with COVID-19 admitted to the National Center for Global Health and Medicine (NCGM) in Tokyo, Japan, from May 22 through June 29, 2021. As part of their daily patient care, we conducted one-on-one individual interviews with inpatients with COVID-19. Each interview lasted approximately 20 minutes. The participants were COVID-19 patients aged 20 years or older who were admitted to the NCGM from May 22 to June 29, 2021, excluding those who had a clear source of infection (*e.g.*, close contact or cluster infection) at the time of admission and those with whom it was difficult to communicate. They were asked to fill out a behavioral questionnaire (Appendix 1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=26*) (*3*) during hospitalization, and based on the questionnaire, interviews were conducted by four researchers (SH, SM, NF, TT) based on an interview guide (Appendix 2, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=26*) from June 10 to July 1, 2021. The researchers were two infectious disease physicians and two infection control nurses. The physicians were involved in COVID-19 patient care, while the nurses were not.

The following items were reviewed: patient demographics (age, sex, nationality); whether COCOA (a contact tracing app) was installed; if installed, whether COCOA notification was given; behavioral history/contact history from 14 days to 1 day before the onset of illness and the location and date of the contact; mask-wearing behavior; and the contact situation in detail. Participants' behavioral history/contact history

was classified into two categories: known high-risk situations and no known high-risk situations. Known high-risk situations included travel by ferryboats or long-distance buses; attending gyms, indoor music events, nightclubs, standing parties, karaoke, indoor dining, or other types of poorly ventilated, enclosed indoor gatherings; and a history of staying in endemic areas (either domestic or international) (*3*). Particular attention was paid to situations in which face-masks were removed, particularly during the 4 to 7 days before the onset, which is the most likely window of exposure based on the incubation period (*4*). Next, the number of participants who were in situations with a known high risk of infection was also tabulated by the situation. We then conducted a qualitative analysis of the information given during the interview regarding patient's thoughts and beliefs about the possible sources of infection. The study protocol was reviewed and approved by the Ethics Committee of the Center Hospital of the NCGM after compliance with the condition that a document that declares an opt-out policy by which any potential participant and/or their relatives could refuse to be included in this study was uploaded on the website of the Center Hospital of the NCGM.

Among the 43 COVID-19 patients aged 20 years or older who were admitted to the NCGM Hospital during the study period, 29 were included, excluding nine patients with a clear source of infection at the time of admission, three patients on a ventilator, one patient who was difficult to interview because of his general condition, and one patient who was difficult to interview because of a language barrier. Among the 29 patients, 23 were interviewed. Three patients were discharged from the hospital prior to the interview, and three were not interviewed immediately after admission. Among the 23 participants, one incorrectly stated the date of onset as the date of hospitalization, and the interview was therefore considered invalid. The responses of the 22 participants were analyzed.

The risk of infection based on the behavioral/contact histories

Among the 22 participants, 17 (77%) were male and five (23%) were female; the median age was 52.5 years (interquartile range: 44–66 years), and 19 (86%) were Japanese. Six of the 22 participants (27%) had downloaded the COCOA notification app, none of whom had received a notification. The behavioral/contact histories of the participants were categorized into situations with known high risk of infection and the others, as shown in Table 1. We classified the behavioral/contact histories into two categories: We categorized them into two groups: those with a high risk of infection (n = 24), including indoor dining (n = 21), attending an indoor music event (n = 1), or attending gym (n = 2), and those with a low risk of infection (n = 118), including shopping (n = 34), working (n = 22), using public transportation (n = 31), visiting medical institutions (n = 14), leisure activities (n = 6), and others (n = 11). Among the 24 high-risk situations, 21 (88%) were related to indoor dining, and masks were not worn in 22 situations (92%).

With regard to the participants who were in known high-risk situations, 12 out of 22 (55%) reported that their activities involved eating or drinking. Of these, six reported having dinner with multiple people, including friends, and of these, five had dinner at a restaurant, three at a pub or a bar, one at a birthday party with more than 20 people, and one at a dinner party using a delivery service (with some participants reporting several of these activities). In addition, one person attended an indoor music concert without wearing a mask, one person used a gym, and one person was a gym instructor. There was one person overlapping with the 12 participants whose activities involved eating and drinking.

Examples of thoughts and beliefs of the patients who thought they could contribute to infection, include: "I don't think that I need to wear a mask while traveling except by train or at work"; "I think that it is okay to eat while chatting in the company cafeteria"; "I think that it is okay for staff to talk to each other without masks after work"; "I feel that it is not good, but I don't want to wear a mask at a concert venue"; "I didn't know that eating out was a risk for infection"; "It was a private barbershop, so there was no need to wear a mask"; and "If it was a private golf game, there would be no need to wear a mask even if there were four of us".

Enhancing risk awareness and promoting infection prevention

One of the most important findings in this study was that masks were not worn in 22 (92%) of the 24 known high-risk infection situations. In addition, 14 of the 22 participants (64%) had a history of known high-risk infection behaviors. With regard to patients' thoughts and beliefs that could contribute to infection, the participants had opportunities to become infected in well-known high-risk situations. This suggests that participants had a low level of awareness of infection control or a lack of knowledge about infection control. Since this survey was conducted after the fourth wave, decreased interest in infection risk information, decreased concern, and optimism bias (*5*) may have contributed the infections. There is a concern about the spread of COVID-19 variant (*6*), it is necessary to consider effective countermeasures for providing accurate information and fostering risk awareness so that they can lead to infection prevention measures.

The second important finding was that there were situations where three participants were teaching karaoke, met a friend who tested positive, and had a one-on-one lesson with a gym instructor with their masks

Table 1. Possible sources of exposure based on behavioral histories from 14 days to 1 day before the onset

Activity type	Activity	*n*
Known high risk		
Indoor dining		21
	Eating out with friends	3
	Ramen shop	3
	Soba shop	2
	Izakaya with friends	2
	Chinese restaurant	2
	Chinese restaurant	1
	Izakaya with friends	1
	A birthday party at a pub with close contact with about 30 people	1
	Japanese restaurant	1
	Company cafeteria	1
	Eating out with friends	1
	Eating out alone	1
	Eating out alone	1
	Order a home delivery service and have a meal with 4 people	1
Indoor music events		1
	Attend a piano concert with vocalists	1
Gyms		2
	Use of fitness club	1
	Instructor	1
Others		
Shopping		34
	Shopping at supermarkets	8
	Convenience store	8
	Shopping at supermarkets	5
	Convenience store	3
	Shopping at department stores	2
	Florist	2
	Convenience store	1
	Convenience store	1
	Shopping at department stores	1
	Real estate agent	1
	Pharmacy	1
	Bicycle shop	1
Workplace/work-related		22
	Meetings and interviews at the workplace	10
	1–2-hour conversation with colleague after work – colleague tested positive	3
	Meetings and interviews at the workplace	2
	Teaching karaoke	1
	One-on-one lessons as an instructor	1
	Office work at a facility for the disabled	1
	Cleaning the bullet train	1
	Transportation of users of the facility	1
	Serving customers at cafe	1
	Brushing teeth in the bathroom at work	1
Public transportation and transportation-related		31
	Bicycling	8
	Commute by train	5
	Bicycling	4
	Commute by foot	4
	Commute by train	3
	Commute by foot	2
	Commute to work	2
	Commute by train	1
	Commute by foot	1
	Commute by cab	1
Medical institution		14
	Clinic	5
	Vaccination site	3
	Hospital	2
	Hospital	1
	Clinic	1
	Dentist	1
	Dentist	1
Leisure		6
	A walk	3
	Golf with family and friends	2
	A walk in the red-light district	1
Others		11
	Use of delivery service	2
	Bank	2
	Bank	1
	Meeting with a friend who tested positive	1
	Hotel (cleaning staff's work was messy)	1
	Barbershop	1
	Barbershop	1
	Visit of the glass replacement company	1
	Visit to relative's house	1

Places where the mask was removed are underlined. Activities that took place 4 to 7 days before the onset are in **bold**.

on. Even if a mask is worn, it is not 100% effective at preventing infection (*7*).

The third important finding was that there were situations in which two participants did not wear a mask during dental procedures and in barber shops. It is possible that the risk of infection was increased due to the lack of mask use, but it is unclear whether the participants actually became infected through these activities. In this study, we were not able to identify any new source of infection that had not been previously identified.

This study had several limitations. First, we examined the relationship between the participants and interviewers. Two of the interviewers were physicians in the department of infectious diseases who had treated some of the participants, and this may have affected their responses during the interview. Second, there was recall bias. Third, there is a possibility that some of the participants intentionally did not report situations in which there was a known high risk of infection. Fourth, only one interview was conducted with each participant, and the content of the participants' answers was not reconfirmed. Fifth, this study was not a prospective qualitative study, but a retrospective chart analysis. Thus, the sample size was small and the qualitative information might not be saturated. Sixth, it is not known whether participants wore masks appropriately, because this was not verified. Last, the percentage of infections caused by the Delta variant ranged from 1.5% to 31.6% during the survey period (*8*), and the survey was conducted at a time when the proportion of infections due to the Delta variant was relatively lower than during the fifth wave.

The urgency of providing accurate information and raising risk awareness on infection prevention and control of COVID-19 in the absence of identified new sources of infection

When the number of newly infected persons in the fourth wave showed a decreasing trend, we investigated the sources of infection of newly infected persons. In situations with a high known risk of infection, many of the cases were related to eating and drinking, and we did not identify any new sources of infection. In situations with a high known risk of infection, insufficient use of masks was evident, and there was a low level of awareness and lack of knowledge about infection control. Providing accurate information and raising risk awareness on infection prevention and control of COVID-19 is urgently needed.

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LETTER

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COVID-19 and liver surgery in France, Italy, Japan, and the United States: A report of a single topic conference of Eastern and Western Association for Liver Tumors (EWALT) 2021

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Abstract: The Eastern and Western Association for Liver Tumors (EWALT) is an international association for clinical and basic scientists dedicated to worldwide study on hepatocellular carcinoma. A single topic conference of EWALT on "COVID-19 and Liver Surgery" was held online on April 23th, 2021. The presenters from France, Italy, Japan, and the United States, reported the current situation of COVID-19 in each country and the influence on the clinical practice and education in hepato-pancreato-biliary surgery. Here we would like to summarize the core of this single topic conference.

***Keywords*:** COVID-19, liver surgery, HPB surgery, EWALT

The Eastern and Western Association for Liver Tumors (EWALT) is an international association for clinical and basic scientists dedicated to worldwide study on hepatocellular carcinoma. A single topic conference of EWALT on "COVID-19 and Liver Surgery" was held online on April 23th, 2021.

Opening remarks by Prof. Norihiro Kokudo, the chairperson of the EWALT 2021 single topic conference, declared the conference open and presented the conference agenda. After briefly touching on the history of EWALT, he expressed his regret that the usual EWALT congress for 2021 had been postponed. Instead of the usual conference, a single topic conference was held online to discuss the theme, "COVID-19 and Liver Surgery". Prof. Kokudo remarked that he was pleased with the large number of participants from numerous countries (163 participants from 21 countries) who registered for the conference. In addition, the upcoming EWALT congress, re-scheduled for February 2022, was announced.

A video message from Prof. Masatoshi Makuuchi, a co-organizer of the first EWALT conference, was broadcast. He thanked the participants for attending the conference and hoped that the discussion of "COVID-19 and Liver Surgery" during the conference would contribute to the fight against COVID-19 in countries around the world. He also expressed his hopes of seeing participants in person at the next conference in February 2022.

As the Keynote lecture, Prof. René Adam, the president of the Medical Board of the Paul Brousse Hospital, reported the results of international surveys and studies on surgery during the COVID-19 pandemic (Figure 1). An online survey conducted by the European-African Hepato-Pancreato-Biliary Association (E-AHPBA) Scientific & Research Committee during the first wave of COVID-19 revealed that most medical facilities experienced limited use of operating rooms, a delay in cancer surgery, and cancelation of non-essential surgery; this was especially true in countries with a higher incidence of COVID-19 (*1*). Another survey, based on the IHPBA-COVID Registry, reported that COVID-19 was associated with a high mortality rate after hepato-pancreato-biliary (HPB) surgery (29% for pancreaticoduodenectomy, 15% major hepatectomy, and 3% cholecystectomy). Lastly, Prof. Adam described a study conducted by Khonsari *et al*. regarding the effects of the COVID-19 pandemic on surgical outcomes (*2*). The study found that the incubation period, a recent history of infection, and a potential nosocomial infection were associated with pulmonary complications and mortality. Prof. Adam described how several studies on liver transplantation reported a higher risk of COVID-19 in transplant candidates and a higher COVID-19-related mortality in candidates and recipients (*3*). He concluded that COVID-19 screening with PCR ± CT was recommended for all patients undergoing HPB surgery and liver transplantation and that positivity for

Figure 1. Presentation in the keynote lecture and panel session. Keynote lecture from René Adam **(A)**, panel speech from Jean-Nicolas Vauthey **(B)**, Nobuyuki Takemura **(C)**, Guido Torzilli **(D)**, and the group photo **(E)**.

COVID-19 would be a contraindication for HPB surgery and liver transplantation.

The Panel Session was moderated by Prof. Norihiro Kokudo and Prof. Kiyoshi Hasegawa. In this session, Prof. Jean-Nicolas Vauthey from the MD Anderson Cancer Center described COVID-19 trends in the United States and Texas. Over 30 million people were confirmed to have COVID-19, and over 5.51 million died from COVID-19. Indeed, COVID-19 became the 3rd leading cause of death in 2020 after cardiovascular disease and cancer. However, the situation has improved as vaccination rates have increased, with the US having a rate of 38% and Texas having a rate of 34%. Prof. Vauthey then described the institutional response to COVID-19 at the MD Anderson Cancer Center, including visitor restrictions, temperature screening at all entrances, travel bans and virtual meetings for all employees, COVID-19 testing before surgery, demarcation of floors based on their COVID-19 status, and promotion of telemedicine. As a result, the risk of COVID-19 after surgery was extremely low, with a risk of 0.46% for inpatients undergoing surgery overall and 0.51% for patients undergoing HPB surgery (*4*). In addition, he presented a case-matched analysis of postoperative morbidity comparing a group that recovered from COVID-19 and a control group. The group that recovered from COVID-19 was given a minimal wait time after recovery of 20 days, and there were no differences in postoperative morbidity between the groups, suggesting the safety and feasibility of surgery for patients who had recovered from COVID-19. Lastly, he mentioned several difficulties with training surgical fellows during the COVID-19 pandemic. He also described how the MD Anderson Cancer Center had implemented several measures with a three-pronged focus (clinical, didactics, and research).

Prof. Nobuyuki Takemura from the National Center for Global Health and Medicine (NCGM) described the status of the COVID-19 pandemic in Japan and institutional policies to combat COVID-19. Japan witnessed three spikes in COVID-19 and was experiencing the 4th spike in the spring, but the vaccination rate was still only 1%. To reduce the risk of infection among medical workers and patients, a

PCR test and screening were mandated for all surgical candidates. As a result, only 5 of 2,348 patients (0.2%) tested positive for COVID-19 after surgery. He mentioned other measures adopted at the NCGM, including expansion of dedicated COVID-19 wards, reconfiguration of the intensive care unit into a negative-air-pressure room, and demarcation of operating rooms based on COVID-19 status. He also described the results of a survey on the impact of COVID-19 on surgery. Seventeen hospitals responded to the survey. Ten hospitals (60%) limited elective surgery during the waves of COVID-19, and some surgeries were delayed or cancelled. Lastly, he mentioned several difficulties with surgical training for younger surgeons since fewer surgeries were performed during the COVID-19 pandemic.

Prof. Guido Torzilli from Humanitas University explained the status of the COVID-19 pandemic in Italy. Italy has experienced three waves of COVID-19 thus far, and the waves have been accompanied by a high rate of hospital bed use and ICU bed displacement. At his hospital, 53 cases of COVID-19 were reported among healthcare professionals, with 20.8% (11/53) involving surgeons (*5*). As a result, surgery at his hospital was restricted, and indeed, the number of liver surgeries and pancreatic surgeries in 2020 decreased by 32% and 13%, respectively, compared to numbers in 2019. In addition, a decrease in outpatients and a shortage of blood components occurred during the waves of COVID-19, which also resulted in fewer surgeries (*6*). Prof. Torzilli also described the framework adopted by the Italian Society of Surgical Oncology to cope with the situation, which included prioritization of surgery, protection of central hospitals, a regionally even distribution of surgery, dedicated paths and logistics, and promotion of telemedicine (*7*). Lastly, he mentioned the vaccination rate in Italy. The rate of vaccination with the 1st dose was 20% and that with the 2nd dose was 8%. The current 3rd wave of COVID-19 has been abating as the vaccination rate has increased.

Prof. Kiyoshi Hasegawa from the University of Tokyo, the co-chairperson of the EWALT 2021 single topic conference, delivered the closing remarks. Prof. Hasegawa thanked the speakers and participants. He also mentioned the upcoming EWALT conference, scheduled for February 2022. The regular conference will be held at Ito Hall at the University of Tokyo. Calls for registration and abstract submission were announced.

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LETTER

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Gaps in the civil registration and vital statistics systems of low- and middle-income countries and the health sector's role in improving the situation

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Abstract: Civil Registration and Vital Statistics (CRVS) is an essential administrative system that provides legal identification to all individuals and accurate statistical data of vital events, such as birth and death rates within the population. Globally, CRVS has been considered a priority issue, especially for low- and middle-income countries where the coverage of this system is poor. This may be attributed to factors such as inefficiency of laws, poor inter-ministerial cooperation, and a lack of awareness among people. To address these issues and improve coverage of the CRVS, the health sector could play a key role by acting as an entry point, collecting accurate vital data, and utilizing information from CRVS. However, the function of the health sector in implementing CRVS has not been fully analyzed in most countries. Further investigation is necessary to develop effective measures to strengthen CRVS.

***Keywords*:** Civil Registration and Vital Statistics (CRVS), birth registration, death registration, low- and middle-income countries, health sector

Civil Registration and Vital Statistics (CRVS) is an essential administrative system that maintains a record of the occurrence and characteristics, and produces vital statistical data, of major events within the population (notably, births, deaths, and marriages) (*1*). CRVS provides individuals with documentation that is necessary to establish legal identity and family relationships, make claims of nationality, exercise civil and political rights, access public services, and participate in modern society (*2,3*). In addition, CRVS can provide governments with essential information that can be used to develop, implement, and monitor policies related to public services.

However, on average, the coverage of civil registration globally is currently low. A United Nations Statistics Division (UNSD) report showed that, as of 2017, only 68% and 55% of countries and regions in the world had birth and death registration rates of 90% and above, respectively (*4*). In addition, there were large regional disparities. As described in Figure 1, in the African region that includes many low- and middle-income countries, the percentages of countries with less than 50% coverage or no data of birth and death registration were approximately 40% and 60%, respectively. However, in Europe and North America, where there are many high-income countries, most countries had more than 90% birth and death registration rates. In light of these facts, international aid organizations, United Nations (UN) agencies, and academic institutions have recognized a weak CRVS system in low- and middle-income countries as a global priority issue for development. Several regional initiatives provide support to these countries to strengthen their CRVS. Moreover, the Sustainable Development Goals (SDGs) highlight the importance of CRVS for accurate measurement of health-related indicators. Target 16.9 of the SDG aims to improve the birth registration rate in order to provide basic legal identification, access to justice for all, and to promote peaceful and inclusive societies for sustainable development.

According to Mikkelsen (*5*), the low civil registration rates in low- and middle-income countries may be attributed to the following factors: *i*) Despite existence of a legal framework, the law in these countries is either inefficiently framed and needs to be revised, or its enforcement is inadequate; *ii*) Due to the lack of inter-ministerial cooperation and unclear division of roles, the legal and statistical position of CRVS becomes ambiguous. Therefore, it becomes difficult for each ministry and agency to adopt collaborative measures to strengthen CRVS; *iii*) People may not be able to access the registration office due to physical and socio-economic barriers, even though they may understand the necessity of civil registration; *iv*) People lack knowledge about the purpose, need, and the medium-and long-term benefit of registration. They

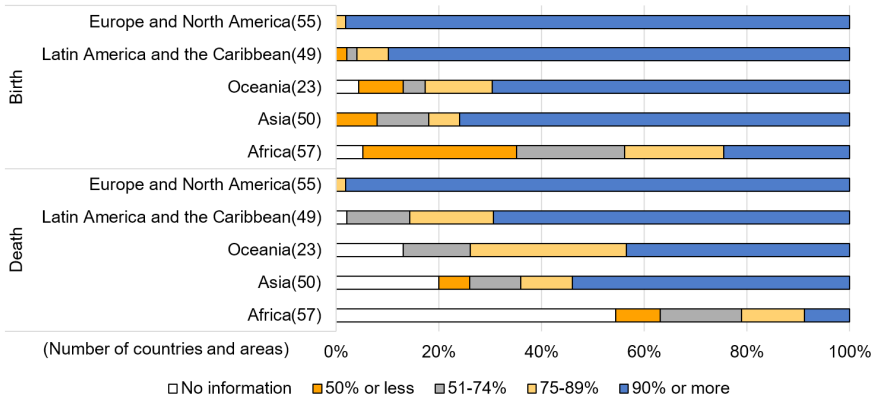

Figure 1. Proportion of countries' live birth and death registration coverage by region. Data Source: United Nations Statistics Division, last updated in UN Data: December 2017 (Original data year 2001-2017), *https://unstats.un.org/unsd/demographic-social/crvs/documents/Website_final_coverage.xls* (accessed October 20, 2020)

therefore have little or no incentive to participate in the registration process.

To address these issues, building an effective collaboration between the health sector and civil registration offices can be helpful. Healthcare institutions can serve as an entry point for the civil registration process and can be used for filing notifications (*1*). For example, in South Africa, a significant improvement was observed in the birth registration rate after registration offices were established within healthcare facilities for childbirth (*6*). Similarly, the health sector can play an important role in improving death registration rates by not only serving as an entry point for registration agencies, but by also identifying the exact causes of death. This information is essential for effective, optimal, and inclusive policy management. In many low- and middle-income countries, it can be difficult to collect accurate information regarding the cause of death. According to Burger *et al*., in about 24% of deaths in South Africa, the reported causes were either ill-defined or unknown, thereby resulting in sub-optimal and biased information for planning purposes (*7*). Therefore, by having the health sectors and clinicians strengthen their ability to create a standard death certificate (*8*) and produce quality medical records, as per the International Classification of Diseases, more accurate and reliable information might be available.

Moreover, the health sector can also use information from CRVS to formulate, implement, and monitor policies for public services to meet the needs of citizens. According to Phillips *et al*. (*9*), a functional CRVS can benefit people's health in the following ways: *i*) By providing the legal foundations for ensuring human rights and access to various social services, and *ii*) by collecting accurate vital and mortality data in a timely manner. Such data can then be utilized by the national or local governments to develop, implement, and monitor health policies in an effective, efficient, and strategic manner. Phillips *et al*. (*9*) further proved that countries with a well-developed CRVS system tended to have significantly longer healthy life expectancies. Thus, the health sector could play a key role in improving CRVS by serving as an entry point and collecting accurate vital data. It could then also utilize information from CRVS to improve health outcomes and formulate policy.

In conclusion, the poor coverage of CRVS in low- and middle-income countries has been globally recognized as a priority issue for achieving the SDGs. Currently, international organizations are taking various measures to strengthen CRVS in collaboration with various stakeholders worldwide. To promote such efforts, a situational analysis of the entire CRVS system was conducted through a rapid survey in the Asia-Pacific region (*5*) and an assessment within the African region (*10*). However, in respect to the health sector, its role in the implementation of the CRVS, its current circumstance, and its possible interventions have not been sufficiently examined. Figure 2 summarizes the abovementioned roles of the health sector in CRVS, the challenges in implementing CRVS reported by Mikkelsen (*5*), and the gaps in the health sector to improve CRVS. In the future, it will be important to conduct subgroup investigations on the functions of the health sector in the entire CRVS system in low- and middle-income countries, in order to translate the global political momentum into concrete actions to improve CRVS throughout the world.

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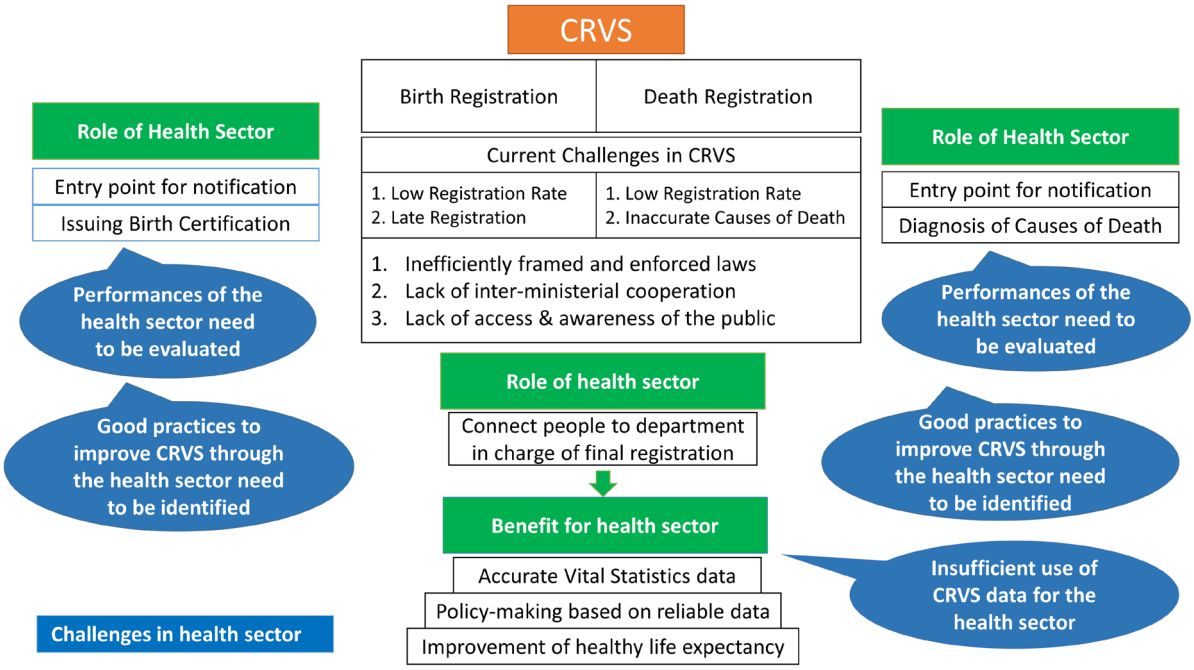

Figure 2. The role of the health sector in CRVS and the existing gaps.

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Advocacy of cyber public health

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Abstract: Thanks to technological advances in computers and communication, the Internet of Things (IoT) has been increasingly used in healthcare to foster digital health in several countries. In conjunction with this trend, cybersecurity has become a matter of paramount importance in terms of protecting healthcare services and health-related information from cyberattacks. With the spread of the COVID-19 pandemic, individuals are encountering false information on the Internet and social media. As a result, an infodemic, which involves people taking inappropriate actions that threaten their health, has occurred worldwide. Cyber public health is a concept that comprehensively encompasses the above issues from the viewpoint of health. This concept helps to prevent various attempts that adversely affect people's health and it utilizes the advantages of cyber technology to promote public health. Cyber public health is also an important concept from the viewpoint of national security.

***Keywords*:** Internet of Things, digital health, cybersecurity, cyberattacks, infodemic, national security

Thanks to technological advances in computers and communication, the Internet has become an indispensable element of social infrastructure, allowing people to exchange images, videos, audio clips, and documents through emails and social media and to purchase products online. With the advent of the Internet of Things (IoT), the concept of digital health is gradually expanding not only in high-income countries but also in low- and middle-income nations (*1*).

The World Health Organization (WHO) has explained digital health as follows: "Digital health, or the use of digital technologies for health, has become a salient field of practice for employing routine and innovative forms of information and communications technology (ICT) to address health needs". The term "digital health" is rooted in eHealth, which refers to "the use of ICT in support of health and health-related fields". Mobile health (mHealth) is a subset of eHealth and is defined as "the use of mobile wireless technologies for health". More recently, the concept of digital health has been introduced as "a broad umbrella term encompassing eHealth (which includes mHealth), as well as emerging areas, such as the use of advanced computing sciences in 'Big Data', genomics, and artificial intelligence" (*2*).

In conjunction with the expansion of digital health, the number of cyberattacks on health facilities is increasing in many countries (*1*). For instance, a hospital system in Germany had to shut down due to a cyberattack, and an emergency room patient died as a result (*3*). Moreover, Japanese research institutes developing vaccines have also reported cyberattacks (*4*). Therefore, the importance of cybersecurity in the field of digital health is increasing. In a white paper on ensuring digital health, the Global Digital Health Partnership describes cybersecurity in digital health as "the means by which health care, better services, and enhanced patient outcomes are delivered and ensured through a resilient and secure digital ecosystem that encompasses culture, people, process, and technology" (*5*).

In addition, the technologies used to conduct cyberattacks can dramatically evolve due to the rapid increase in communication speeds provided by 5G (currently in progress) and threats to conventional cryptography through the development of quantum computing technology (*6*). To enhance its response to cyberattacks, the Japanese Government will establish a cyber defense organization through collaboration among industry, government, and academia in 2022 to analyze cyberattacks and to devise countermeasures (*7*).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) broke out in Wuhan, China in December 2019 and subsequently spread around the world. It has become a crucial public health issue and has negatively impacted the world economy. Governments and institutions have implemented public health measures to control infection rates and prevent the spread of the disease. Several countries – including

Western countries, Japan, China, and Russia – have begun to develop vaccines, medicines, and diagnostic test kits for SARS-CoV-2.

Under such circumstances, a situation known as an infodemic may occur. In response to this issue, the WHO announced a joint statement with the UN, UNICEF, the UNDP, UNESCO, UNAIDS, ITU, UN Global Pulse, and the IFRC on September 23, 2020, about COVID-19 entitled "Managing the COVID-19 infodemic: Promoting healthy behaviors and mitigating the harm from misinformation and disinformation" (*8*).

The joint statement describes infodemics as follows: "An infodemic is an overabundance of information, both online and offline. It includes deliberate attempts to disseminate wrong information to undermine the public health response and advance alternative agendas of groups or individuals. Mis- and disinformation can be harmful to people's physical and mental health; increase stigmatization; threaten precious health gains; and lead to poor observance of public health measures, thus reducing their effectiveness and endangering countries' ability to stop the pandemic" (*8*).

A large number of deaths due to drinking methyl alcohol in Iran and Kazakhstan have also been reported as infodemic-related harms due to COVID-19 (*9*).
Moreover, a threatening new technology – deepfakes – can promote infodemics. Deepfake technology has begun to be used as artificial intelligence has evolved. According to the British Government, deepfakes can be defined as visual and audio content that has been manipulated using advanced software to change how a person, object, or environment is presented (*10*). At the same time, the US–China conflict has heated up, social divisions have expanded inside countries and beyond, and the spread of COVID-19 has spurred economic disparities; consequently, the number of people in dire straits has increased rapidly. As a result, social factors that encourage cyberattacks and infodemics have become more prominent.

Within this context, cyber public health is a concept that comprehensively encompasses the above issues from the viewpoint of health. This approach will help prevent various problems that adversely affect people's health in the cyber realm and utilize the advantages of cyber technology to promote health. Cyberattacks enable hackers to rapidly attack a large number of targets at the same time, at a low cost. Such attacks can negatively affect people receiving medical care at health facilities, cause misunderstandings regarding access to medical services, and hamper the research, development, and production of medical products. Hence, the concept of cyber public health is also important from the viewpoint of national security.

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