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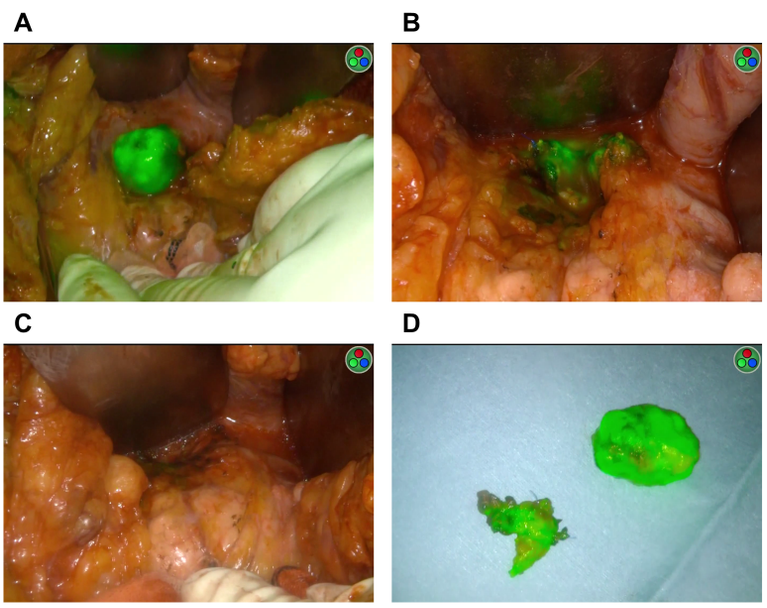

Intraoperative use of ICG fluorescence imaging in HCC (page 407)

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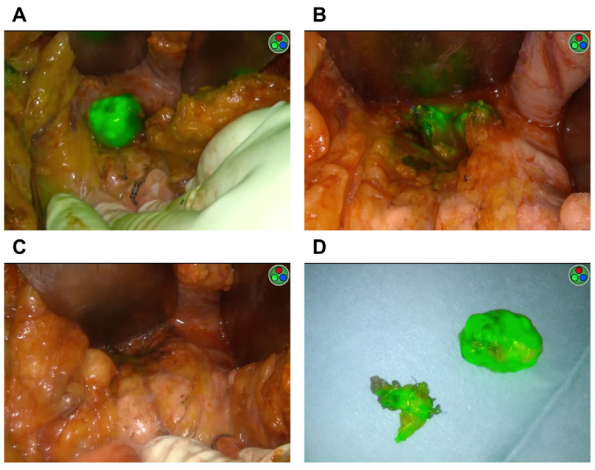

ICG fluorescence imaging using the PINPOINT® camera system. (A) A swollen LN with a strong fluorescent signal. **(B)** After the resection of the swollen LN, the strong fluorescent signal remained. **(C)** After the additional resection, no fluorescent signal was detected. **(D)** ICG fluorescence image of the resected specimens. ICG: Indocyanine green. (page 407)

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EDITORIAL

HIV testing in COVID-19 pandemic and beyond in Japan

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Abstract: In Japan, HIV testing has been offered anonymously and free-of-charge at local public health centers, together with pre- and post-test counseling since 1993. Since then, the number of HIV tests increased steadily to reach a peak in 2008 but has since decreased by 30% during the last decade. The number of tests further decreased in 2020 during the COVID-19 pandemic and steeply by 50% this year compared with the previous year, mostly due to a shift in the workload at these centers to COVID-19-related services. To deal with this decline and thinking beyond the current pandemic, more options for HIV testing are needed, such as self-testing/postal delivery of dried blood spot specimen, a method that is yet to be approved in Japan, in addition to the conventional plasma/serum-based HIV testing.

***Keywords*:** HIV testing, COVID-19 pandemic, self-testing, dried blood spot, postal service

The standard HIV testing protocol in Japan consists of two consecutive reactive tests to confirm an HIV positive diagnosis (*1*). It recommends the use of the 4th generation HIV-1/2 antigen-antibody screening assay followed by another HIV-1/2 antibody confirmatory assay and/or nucleic acid amplification test (NAAT) to detect HIV in serum or plasma specimens. This protocol is employed at laboratories in medical facilities or local public health centers across Japan. At medical facilities, all high-risk individuals (defined as those with incident sexually transmitted infections and multiple sexual partners) should undergo screening for HIV infection. However, many patients with undiagnosed HIV infections are seen several times at medical facilities before they actually undergo HIV testing. In other words, physician-directed testing is not always efficient.

Another opportunity is voluntary testing at local public health centers. Testing at these centers is strongly recommended because it is free of charge, anonymous, and bundled with pre- and post-test counseling. In Japan, such centers have served as voluntary counseling and testing centers for HIV since 1993. The number of HIV tests conducted at such centers reached a peak (at 177,000) in 2008. Admittedly, some of these centers are not always convenient, for example, operating only one hour a week, closed over weekends, requiring appointments, or multiple visits (one for testing and another for results). Therefore, the number of such tests fell by 30% in the last decade (*2*). More importantly, the number of tests conducted in 2020 decreased precipitously by around 50% compared with the previous year (from 142,000 in 2019 to 69,000 in 2020) (*2*) mainly due to the shift in the workload at these centers towards providing COVID-19-related services. To counter this fall in HIV testing, it is important to design alternative methods for HIV testing that can be executed without the need to visit the local public health centers.

The number of self-collected dried blood spot (DBS) test cards delivered by postal service has been increasing steadily in the last two decades, and probably now outnumbers the tests conducted at the local public health centers. The estimated number of such tests is more than 100,000 per year, although the exact number is unknown. The advantages of HIV testing using mail-posted-DBS include simple and easy self-preparation of finger-prick-blood spots at ambient temperature that can be posted 24 hours a day/7 days a week throughout the year. Therefore, people would not need to visit medical facilities or local public health centers. However, DBS is not yet approved as a clinical sample for HIV testing in Japan. In this regard, even in the US, only a limited number of commercial DBS-based test kits have been approved so far by the Food and Drug Administration (FDA) (*3*). To use DBS widely, we have to elucidate its applicability for HIV testing, and current studies are very limited (*4*). According to our data, DBS sample is around 200 times more dilute because a small amount of whole blood on filter paper is eluted with phosphate buffered saline. Therefore, HIV testing with DBS is estimated to be less sensitive than with a plasma sample. Notably, however, the antibody titers are continuously changing over time (*5*) and testing with DBS can detect many HIV patients with enough sensitivity and specificity, excluding those with acute infection. In this context, our previous outreach study (*6*) confirmed the high successful rate of HIV testing with self-collected postal service-delivered DBS in Japanese men who have sex with men (MSM) (representing a high-risk population).

DBS seems feasible and reliable.

When we look at the world, the importance of HIV testing has been considerably recognized in the last two decades and there are currently many options. Advances and innovation in medical technology have helped spread the use of HIV testing in both the developed and developing countries in terms of clinical specimens (*e.g.*, oral fluid or DBS), testing protocols (*e.g.*, self-testing or using postal service), and access to testing (*e.g.*, collaboration with groups or social networking). For maximum reduction in the number of new cases of HIV infection with a goal of "Ending the HIV Epidemic", we have to identify all HIV infected people who are not yet diagnosed, start antiretroviral therapy (ART) as early as possible, and ensure that the viral loads are maintained below the detection levels, based on the scientific concept of "Undetectable equals Untransmittable (U = U)" (*7*). HIV testing is the gateway to a series of steps for expansion of ART coverage. The WHO recently issued innovative HIV testing recommendations in response to contemporary needs (*8*). The US Center for Disease Control (CDC) also recommends repeated HIV testing of key populations, preferably every 3 to 6 months for early assessment of HIV status (*9*). Furthermore, HIV pre-exposure prophylaxis (PrEP) has been offered to high-risk populations worldwide in this decade and a recent study confirmed that PrEP successfully prevents HIV infection in the long term (*10*). People on PrEP are strongly recommended to undergo quarterly HIV testing. Under these circumstances, self-testing using home collection kits or oral swab-based tests should be considered, especially when facility-based services or in-person contact is limited (*11*). Another step towards achieving "Ending the HIV Epidemic" would be the simultaneous implementation of both ART to patients and PrEP to high-risk populations.

Looking back and thinking ahead of the post-COVID-19 pandemic in Japan, we need more "user-friendly" options for HIV testing that allow self-testing, including the use of DBS or oral-swabs as an alternative to plasma or serum specimens. HIV testing should be positioned as the first step to reducing new HIV infections. This should be used in conjunction with early ART after diagnosis and PrEP for at-risk populations. Otherwise, we will have to deal with the more serious situation of HIV endemic in the future.

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Cardiovascular disease, a major global burden: Epidemiology of stroke and ischemic heart disease in Japan

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Abstract: Japan ranks the highest globally, in terms of longevity. The average life expectancy was 81.4 years for men and 87.5 years for women in 2019. Such success in health is attributable to the substantial reduction in age-standardized mortality from cardiovascular diseases, especially stroke (136 per 10^5 in 1980 and 24 per 10^5 in 2015), when stroke mortality was the highest in the world between the 1960s and the 1990s. On the other hand, ischemic heart disease mortality was the lowest in the world between the 1960s and the 1980s and has continued to decline (40 per 10^5 in 1980 and 17 per 10^5 in 2015). Such a disease profile (larger burden of stroke compared to ischemic heart disease) was observed not only in Japan but also in some countries in central Asia and Africa, where small vessel disease (arteriolosclerosis) is assumed to be more common than large vessel disease (atherosclerosis). Between 1970 and 2015, a large decline in the population with high blood pressure levels was observed for both men and women. Meanwhile, there was a moderate decline in the smoking rate among men, and an increasing trend in serum cholesterol levels in both men and women. The sharp and extensive socioeconomic development between the 1960s and 1990s contributed to these health outcomes, while preventive measures and improved emergency medical care also contributed to the reduction of risk factors, disease incidence, case-fatality, and mortality. However, there is a threat of increasing incidence of ischemic heart disease in urban male employees and middle-aged male residents. Japan, with a super-aging society, needs to develop a new model for the prevention and control of cardiovascular disease and related health issues, with emphasis on efforts towards the early (primordial) prevention of cardiovascular disease as well as the attenuation of their progress towards chronic heart failure, chronic kidney disease, and vascular dementia.

***Keywords*:** cardiovascular disease, epidemiology, pathology, prevention, Japan

Introduction

Cardiovascular disease (mainly stroke and ischemic heart disease) is a major non-communicable disease, accounting for over two-thirds of total mortality worldwide (*1*). Cardiovascular disease is an emerging serious health issue in mid-to low-income countries as well as in high-income countries (*2*). However, the health burden of cardiovascular disease depends on the disease profile (the dominance of ischemic heart disease, stroke, or both diseases) as ischemic heart disease and stroke have differential demography (*3-5*), pathology (*6,7*), and clinical consequences (*8*).

There has been a steep decline in the mortality from stroke and ischemic heart disease in Japan, with the dominance of stroke compared to ischemic heart disease (2,9). The differences in etiologic factors and pathology attract scientific interest and also provide policymakers with a clue to construct preventive strategies for cardiovascular disease. Our experiences and evidence may be applied to other countries, especially in East Asia and Central Africa, where mortality from stroke is higher than that from coronary heart disease (*1,5*) which could be attributable to differential vascular pathology (*6,7*).

This article reviews the trends for stroke and ischemic heart disease with a look at the background lifestyles, and pathology in Japan compared to other countries.

Trends for mortality from stroke and ischemic heart disease

Japan had two- to three-fold higher mortality from stroke than the US, UK, and France in 1980, according to WHO statistics (Figure 1) (*2*). Between 1980 and 1990, stroke mortality declined more steeply in Japan than in western countries and continued to decline into 2015. On the other hand, mortality from ischemic heart disease in Japan was one-sixth, one-fifth, and half of that in the

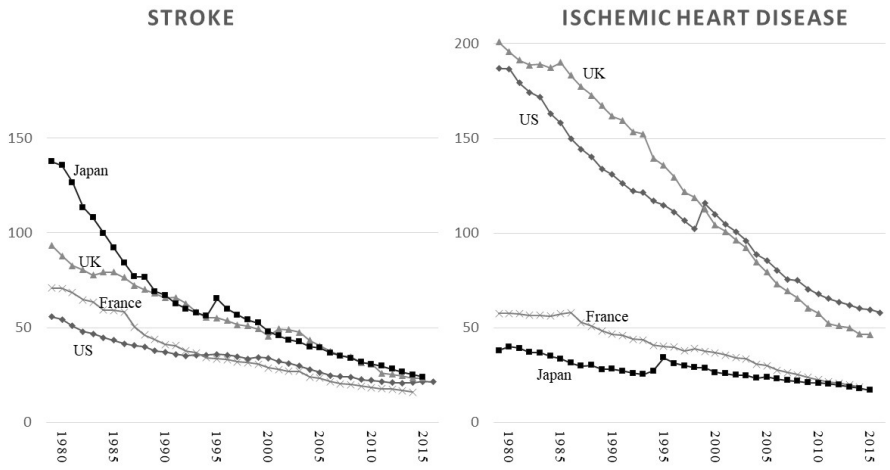

Figure 1. Age- and sex-adjusted mortality rate per 100,000, standardized to the standard WHO population, from stroke (A), ischemic heart disease (B), and stroke relative to ischemic heart disease. Source: Reference 2.

Table 1. Ratios of age- and sex-adjusted mortality rates from stroke and ischemic heart disease (IHD) per 100,000 in selected countries

Year	Japan Ratio	Stroke/IHD	US Ratio	Stroke/IHD	UK Ratio	Stroke/IHD	France Ratio	Stroke/IHD
1980	3.39	135.6/40.0	0.29	54.4/186.7	0.45	87.8/195.7	1.23	70.8/57.5
1985	2.75	92.3/33.6	0.27	43.4/158.1	0.42	79.4/190.1	1.03	59.2/57.3
1990	2.38	67.1/28.2	0.28	37.1/130.9	0.41	65.7/161.6	0.88	41.1/46.5
1995	1.91	65.3/34.1	0.31	35.9/114.7	0.41	55.2/135.7	0.84	33.5/40.1
2000	1.83	48.0/26.3	0.31	33.9/110.0	0.44	45.5/104.2	0.78	28.8/36.8
2005	1.64	39.4/24.0	0.31	26.3/85.5	0.51	40.4/79.3	0.78	23.4/29.9
2010	1.49	30.8/20.7	0.33	22.3/67.7	0.53	30.8/57.6	0.82	18.5/22.5
2015	1.40	24.0/17.1	0.36	21.6/59.5	0.51	23.5/46.3	0.85	16.0/18.8

US, UK, and France, respectively, in 1980, after which the mortality declined substantially. The magnitude of decline was larger in the US and the UK than in France and Japan. It is noteworthy that Japan, with the lowest mortality from coronary heart disease, showed a continuous decline in mortality, with the mortality in 2015 being half of what it was in 1995.

The ratio of stroke and ischemic heart disease mortality between 1980 and 2015, in Japan, was 3.39 in 1980 and 1.40 in 2015 (Table 1). The corresponding ratios were 0.29 and 0.36 in the US, 0.45 and 0.51 in the UK, and 1.23 and 0.85 in France. These findings indicate that the predominance of stroke in Japan has converted to being only moderately higher compared to ischemic heart disease, while ischemic heart disease has remained predominant in the US and UK. France showed trends between those of Asia and the US and the UK.

Kim *et al.* clearly illustrated that a higher mortality for stroke compared to ischemic heart disease, as seen in Japan, was also seen in other Asian countries, such as China, Mongolia, and Thailand; some European countries; and many central and southern African countries, according to the analysis of global variation in the relative burden of stroke and ischemic heart disease (*5*). The geographic differences in stroke and ischemic heart disease mortality suggest variation in pathology, risk factors, behaviors, and socio-economic factors.

Two types of vascular pathology and related factors

Such large differences in the profiles of cardiovascular diseases among countries can be explained by the lower proportion of large vessel pathology (atherosclerosis) and the higher proportion of small vessel pathology (arteriolosclerosis) in Japan than in the US and UK. The prevalence of arteriolosclerosis is probably higher, and that of atherosclerosis is lower, among urban residents than among rural residents of Japan because the incidence of ischemic heart disease was higher and that of stroke was lower in urban residents than in rural residents. For example, the incidence of ischemic heart disease in men aged 40-69 years in 1998 and 2003 was 127 and 65, respectively, and that of stroke was 118 and 231, respectively (*4*).

As shown in Figure 2, atherosclerosis is characterized by lipid accumulation and inflammatory cell proliferation, leading to the formation of 'plaque' and eventually, blood clot, which causes ischemic heart disease and ischemic (large vessel) stroke (*6,7*). High total cholesterol level (from a diet rich in saturated fat, such as meat), diabetes, smoking, and to a lesser extent, hypertension, accelerates this type of pathology. On the other hand, arteriolosclerosis is characterized by the death of smooth muscle cells, which constitute a major component of the vascular wall. This leads to

the weakening of the vascular wall (constructed mainly by smooth muscle cells) and its subsequent rupture, or cell proliferation due to an excessive healing process, that causes intracerebral hemorrhage or ischemic (small vessel) stroke, namely lacunar stroke. Hypertension, and to a lesser extent, diabetes and smoking accelerate arteriolosclerosis. Low total or LDL-cholesterol levels (*10-12*), low intake of saturated fat (*13-15*), and low intake of protein (*13-16*) have been suggested to be linked to this type of pathology and intracerebral hemorrhage. A recent large observational and genetic study of the Chinese Kadoorie Biobank and a meta-analysis of randomized trials supported a causal relationship between lower LDL-cholesterol levels and a higher risk of intracerebral hemorrhage (*17*).

Strong trends for systolic blood pressure levels and stroke mortality

Figure 3 shows that sex- and age-specific mean value of systolic blood pressure, a strong risk factor for stroke, declined substantially between 1970 and 2019 among national representative samples of men and women aged ≥ 30 years (*18*). For both men and women, systolic blood pressure levels have declined substantially in all age categories with a larger decline among older ages than younger ages, and with a larger decline between 1970 and 1990 than thereafter. Meanwhile, age-adjusted stroke mortality peaked in 1965 and declined materially from 1965 to 1990 for both men and women. After 1990, stroke mortality continued to decline although the rate of decline became smaller (Figure 4) (*19*).

Figure 5 illustrates trends for cardiovascular risk

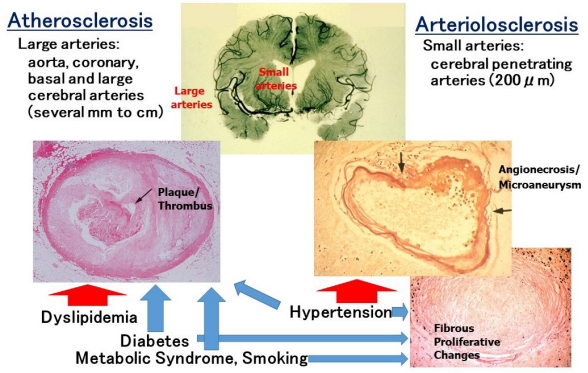

Figure 2. Two types of vascular pathology and related factors, atherosclerosis and arteriolosclerosis. Source: References 7.

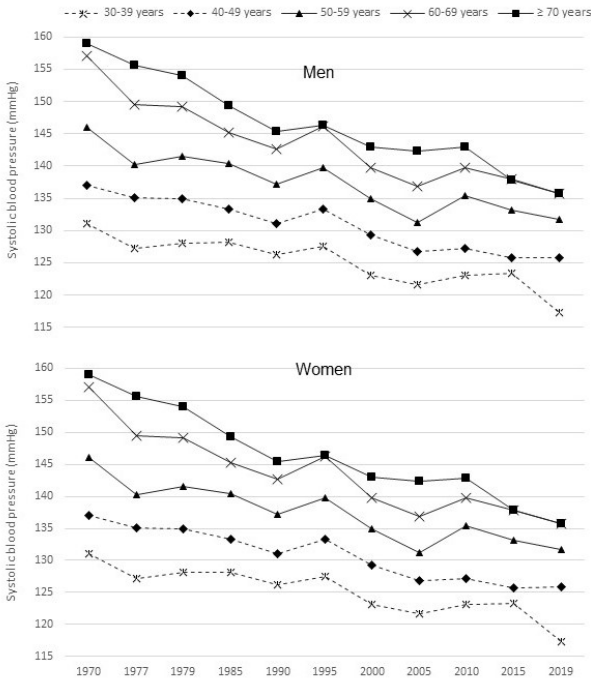

Figure 3. Sex- and age-specific trends for systolic blood pressure levels between 1970 and 2019. Source: National Health and Nutrition Survey, Reference 18.

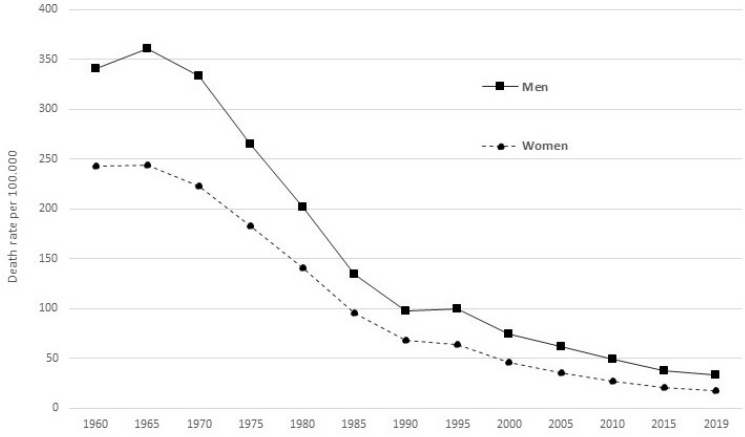

Figure 4. Sex-specific trends for age-adjusted mortality rates per 10,000, standardized to the 1960 Japan national standard population, for stroke between 1960 and 2019. Source: Reference 19.

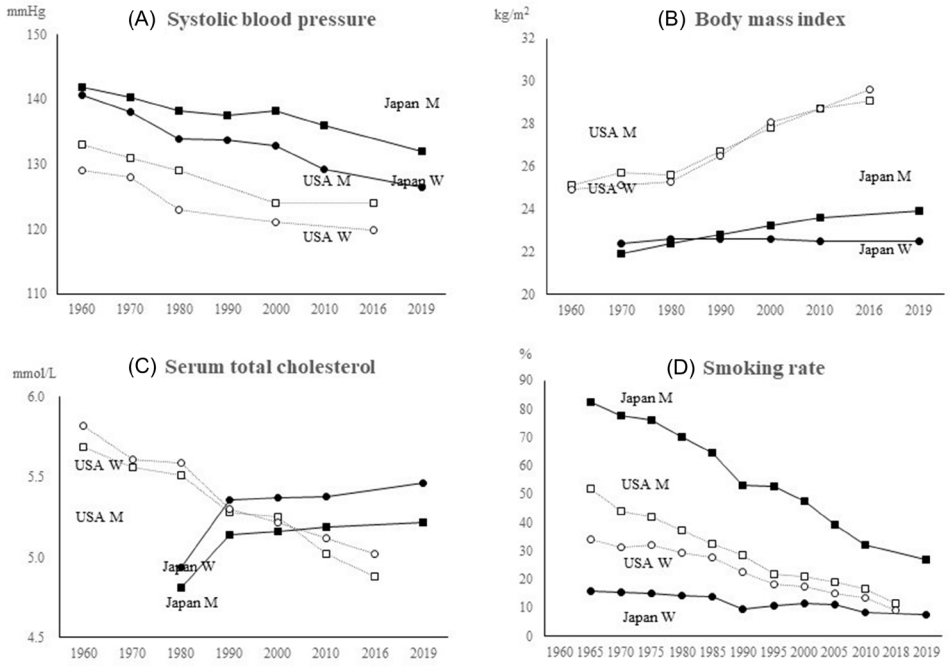

Figure 5. Sex-specific trends for cardiovascular risk factors such as mean levels of systolic blood pressure (A), body mass index (B), serum total cholesterol (C), and smoking rate (D) in national samples of Japan and the US between 1960 and 2019.

factors such as mean values of systolic blood pressure, body mass index (BMI), serum total cholesterol and smoking rate in Japan and the US between 1960 and 2019. Although mean systolic blood pressure levels were higher in Japanese adult men and women than their US counterparts, levels declined substantially in all groups (*18,20,21*). Mean BMI was much lower in Japanese than in Americans throughout the years while mean BMI in Americans has increased continuously since 1980 (*18,22,23*). Mean blood cholesterol in Japanese men and women increased from 1980 to 1990 to match that of their American counterparts, after which they stabilized while American levels continued to decline. Smoking rate was much higher in Japanese men and much lower in Japanese women compared with Americans (*18,24,25*). Although the rate declined substantially it still remains relatively high in Japanese men (*18,26*).

These strong trends for blood pressure and stroke mortality have been attributed to nutritional improvements, such as reduced salt intake, balanced intake of fresh vegetables, fruits, fish, and meat, and the reduction of hard labor due to mechanization (*27*). Increased blood total cholesterol in the Japanese may reflect an increased intake of saturated fat from meat and dairy products (*18*). These lifestyle changes were enhanced by rapid and large economic development between the 1960s and 1990s (*7,8*). The substantial decline in stroke mortality has also been attributed to the improvement in emergency medical care since the late 1970s. A community-based registry reported that the case-fatality rate within one month declined substantially for stroke: 22.0% in 1977-1981, 19.9% in 1982-1986, and 16.5% in 1987-1991 (*28*).

Effects of a community-based stroke prevention program

The question is whether such a large decline in blood pressure and stroke mortality is caused by economic development. The answer is partly yes.

Our research team launched a stroke prevention program in several communities in the early 1960s, after the initiation of the 1961 universal health coverage (*29*). The program consisted of systematic blood pressure screening for the detection of hypertension through annual health check-ups for individuals aged ≥ 30 years, referral of severe hypertensive and other high-risk individuals to local physicians, health instructions for lifestyle modification by physicians, public health nurses, and nutritionists on an individual and group basis, adjunct activities by health volunteers, and community-wide media campaigns for health education (*29*). As shown in Figure 6, the program achieved a larger decline in systolic blood pressure levels by 3 to 4 mmHg, and a larger reduction in stroke incidence by 21% (*32,33*) with a cost-saving effect on public health services (including salaries of health professionals and clerical workers and costs of screening and related health activities) and medical care (treatment for hypertensive and stroke patients) (*31*), compared to a reference community.

A 3.5-year community-based intervention trial in seven cities in China in 1987 demonstrated a program requesting people with hypertension and a history of

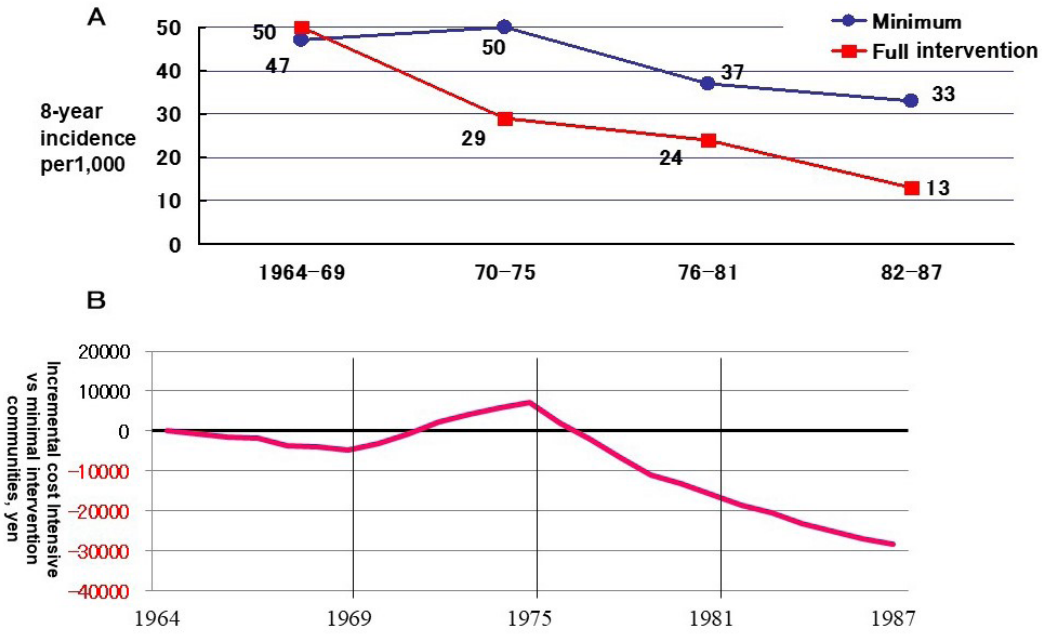

Figure 6. Sex- and age-adjusted 8-year incidence per 1,000 of stroke (A) and incremental cost (yen per capita) (B) in the full intervention versus minimum intervention communities from 1964 to 1987. Source: References 21, 24.

diabetes or heart disease to visit the clinic regularly for treatment (drug treatment and lifestyle intervention including weight reduction, increased physical activity, and modification of dietary sodium and alcohol intake) along with community-based health education. The program was feasible and effective in reducing the incidence of stroke by 31% more than the control cohort (*32*).

The future threat of increasing incidence of ischemic heart disease

What is the future health issue regarding cardiovascular disease in Japan, where mortality from stroke and ischemic heart disease has been declining since the 1960s? The answer is a threat of an increasing incidence of ischemic heart disease in some populations. Trends for ischemic heart disease mortality varied by sex, age, and regions; the decrease in mortality was lesser among men residing in the Tokyo and Osaka metropolitan cities than among those in the rest of Japan (*33*). There was no substantial change in mortality among men aged 30-49 years (around 10 per 100,000), whereas those in the rest of Japan showed a steady decline between 1969-1970 and 1989-1990. This was probably due to a substantial decline in blood pressure levels for both men and women and a moderate reduction in the smoking rate for men, albeit with increased cholesterol levels. The improvement of emergency medical care also contributed to the decline in coronary heart disease mortality, and a prefecture-based registry showed that the case-fatality rate within one month for acute myocardial infarction was 17.0%, 13.1%, 9.1%, and 7.3% in 1980-1984, 1985-1989, 1990-1994, and 1995-1999, respectively (*34*).

Age-adjusted incidence of ischemic heart disease increased from 0.4 per 1,000 person-years between 1963 and 1970 to 1.5 per 1,000 person-years between 1979 and 1986, and plateaued until 1987-1994 among male employees aged 40-59 in Osaka (*35*). A similar trend was observed for Osaka male residents aged 40 to 69 years, with an increased incidence per 100,000 person-years from 56 in 1980-1987 to 127 in 1996-2003 (*4*). In contrast, the incidence of ischemic heart disease remained low and did not change among female residents in Osaka (from 1,336 to 23 per 100,000 person-years) (*35*). The increasing incidence of ischemic heart disease in middle-aged men is probably due to increased blood cholesterol levels, physical inactivity, and reduced intake of fish (*8*).

Conclusions

In the face of a super-aging society (*36*), Japan needs to develop a new model for the prevention and control of cardiovascular diseases and related health issues. We need to make efforts in public health services and medical care towards the early (primordial) prevention of cardiovascular disease, as well as the attenuation of their progress towards chronic heart failure, chronic kidney disease, and vascular dementia. To enhance early prevention, data from pre-birth to old age, segregated among different administrative sections are urgently required. Nevertheless, our success in cardiovascular health so far provides valuable clues for other countries with a rapidly aging population, especially in Asia and the mid-East (*37*).

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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Endoscopic resection for gastrointestinal tumors (esophageal, gastric, colorectal tumors): Japanese standard and future prospects

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Abstract: Endoscopic resection (ER) techniques such as polypectomy, endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) are widely accepted as a less invasive treatment for gastrointestinal (GI) tumors. Since there is a limit to the size that can be resected by EMR and it is often divided, it is not possible to accurately evaluate the degree of cancer progression, and the cancer remains or causes recurrence. ESD is a technology that overcomes these weaknesses. ER techniques are considered for tumors that have a very low possibility of lymph node metastasis and are suitable for en-bloc resection. As ESD became more widespread, the difficulty of treating ESD was gradually resolved by the development of technology and equipment, the curative resection rate increased, and the complication rate decreased. ER techniques have become the standard treatment for early cancer and precancerous lesions in Japan, and the therapeutic indications are expanding day by day. The indications for whether endoscopic treatment can be performed are defined by the guidelines for each organ such as the esophagus, stomach, and colorectum. In the coming aging society, it is also necessary to evaluate the indications for endoscopic treatment and invasive treatment. In addition, recent advances in endoscopic technology are making it possible to remove submucosal tumors that previously required surgery. In this review, we summarize the recent Japanese standard indications of ER for each GI location and future prospects of ER.

***Keywords*:** endoscopic mucosal resection, endoscopic resection, endoscopic submucosal dissection, gastric cancer, esophageal cancer, colorectal cancer

Introduction

The endoscopic resection (ER) techniques of gastrointestinal (GI) neoplasms are very beneficial for patients because of its low invasiveness and low risk of complications. Polypectomy, endoscopic mucosal resection (EMR), and endoscopic submucosal dissection (ESD) are the endoscopic treatment methods of ER. ESD has been approved by the Ministry of Health, Labor, and Welfare of Japan for the stomach as the earliest since 2006, followed by esophagus in 2008 and colorectum in 2012, and is now the standard nationally approved insurance treatment. However, ER adaptation is limited because ER is considered for tumors that have a very low possibility of lymph node metastasis and are suitable for en-bloc resection. The indications for whether endoscopic treatment can be performed are defined by the guidelines for each organ such as the esophagus, stomach, and colorectum. It is necessary to make an accurate preoperative diagnosis in order to perform treatment according to the indications (*1-3*). Preoperative diagnosis is usually performed with endoscopic observation, dying, image-enhanced endoscopy, and endoscopic ultrasonography.

This article gives an overview of the current Japanese state of ER, recently developed technologies, and desired technologies for the future.

Methods of endoscopic resection (ER)

Polypectomy

In this technique, a snare is placed on the stalk of the lesion, and the lesion is electrocauterized using a high frequency current. This method is mainly used for protruding lesions.

Endoscopic mucosal resection (EMR)

In this technique, the lesion is elevated by local injection of a liquid such as physiological saline into the submucosa. The lesion is electrocauterized the same as in the case of polypectomy.

Endoscopic submucosal dissection (ESD)

In this technique, the lesion is elevated by local injection of a liquid such as sodium hyaluronate solution

into the submucosa of the perilesional area; then, circumferential incision of the mucosa surrounding the lesion and dissection of the submucosa is performed with a high-frequency electric knife.

The principles of indication for ER

ER is considered for tumors that have a very low possibility of lymph node metastasis and are suitable for en-bloc resection.

Esophagus

i) *Treatment of squamous cell carcinoma. Absolute indication*: cancer infiltration remains up to the lamina propria (EP, LPM), cancer involving less than three-quarters of the esophageal circumference. *Expanded indication*: cancer depth reaches the muscularis mucosae, stays within the submucosal layer 200 μm. If mucosal resection occupies more than three-quarters of the lap, stenosis is expected to occur after resection, so sufficient discussion and prevention of stenosis are required (*4,5*).

ii) *Treatment of Barrett's cancer.* It is similar to that of squamous cell carcinoma. ER is indicated for those whose wall depth remains within the lamina propria (EP [remains in the epithelium (non-invasive lesions)], SMM [remains in the superficial muscularis mucosae], and LPM [does not reach the deep muscularis mucosae]) (*6*).

iii) *Criteria for additional treatment after ER of esophageal tumors.* If tumor was pT1a-MM with positive vascular invasion or pT1b (200 μm or more), additional treatments, such as surgical resection and chemoradiotherapy, are strongly recommended because of the high metastasis rate (*6*). Argon plasma coagulation (APC) and photodynamic therapy (PDT) are considered as additional treatment for residual marginal lesions for cases with difficulty in raising the mucosa during additional ER after radiation therapy, and cases with bleeding tendency (*6,7*).

iv) *Follow-up observation after endoscopic treatment of esophageal tumors.* It is reported that local recurrence often occurs within 1 year after the initial treatment. Long-term follow up is required because lymph-node recurrence or organ recurrence may be detected 2-3 years later. When it comes to occurrence of stenosis, it is strongly recommended to perform prophylactic balloon dilatation, local steroid injection, or oral steroids to prevent stenosis after endoscopic treatment of esophageal cancer (*8-10*).

Gastric cancer

i) *Absolute indication*: differentiated-type adenocarcinoma without ulcer (UL), differentiated-type adenocarcinoma with UL, diameter ≤ 3 cm.

ii) *Expanded indication*: undifferentiated, mucosal cancer ≤ 2 cm without UL.

iii) *Criteria for additional treatment after ER of gastric cancers.* Figure 1 shows the criteria of indication and curability for ER of early gastric cancer (EGC) in the Japanese Gastric Cancer Association (JGCA) guidelines version 5, which were performed in 2018 (*11*). eCuraC is equivalent to conventional non-curative resection, with careful follow-up or additional treatment for lesions in C-1 and additional surgical resection in C-2. It has been reported that 17-29% of patients with ER will have endoscopic non-curative resection (*12-15*).

iv) *Follow-up observation after ER of gastric cancers*. Follow-up with annual or biannual endoscopy is recommended for curative resection. *Helicobacter pylori* eradication is recommended for all cases because eradication therapy reduced the annual incidence of

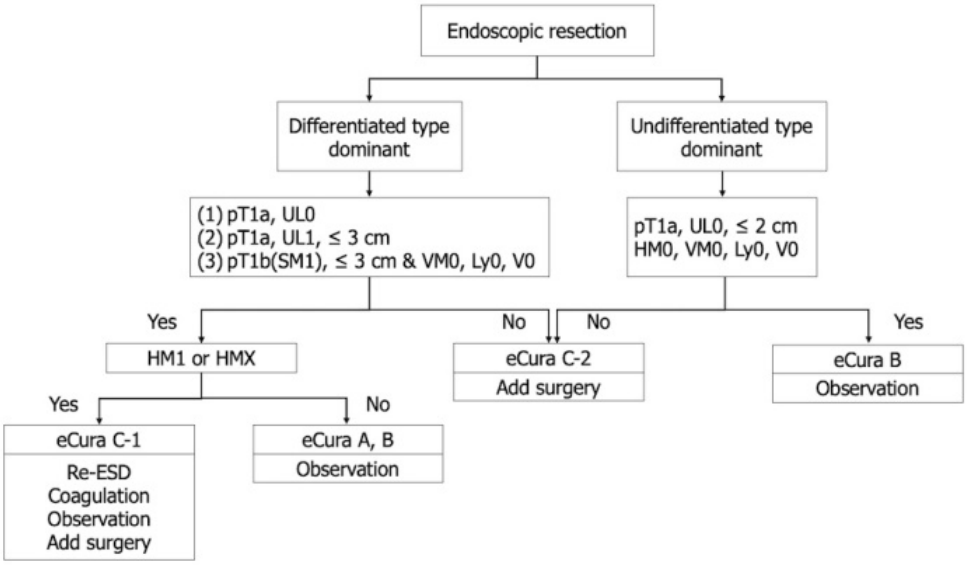

Figure 1. Criteria of the indication and curability for ER of EGC in the JGCA guidelines version 5 (*11*)**.** ESD: endoscopic submucosal dissection; EMR: endoscopic mucosal resection; M: intramucosal invasion; SM1: mucosa Underlayer invasion (less than 500 μm from mucosal muscle plate); SM2: submucosal invasion (more than 500 μm from mucosal muscle plate); UL, ulcer.

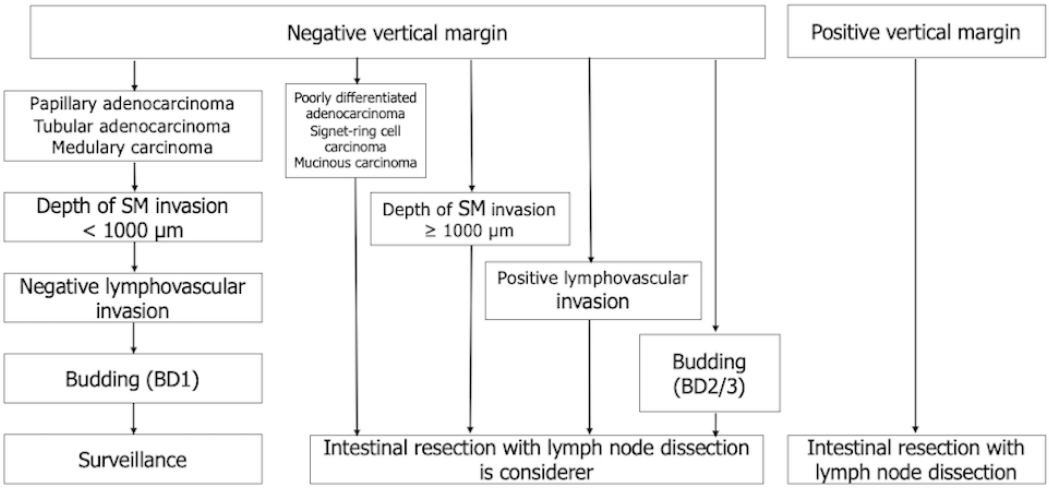

Figure 2. Treatment strategies for pT1 colorectal cancer after endoscopic resection (*2*).

metachronous gastric cancer (*16*). However, some studies showed that eradication therapy after ER had no impact on the development of metachronous cancer (*17,18*). Further investigations are needed on this issue.

Colorectal tumors

i) *Indication criteria*: early colorectal cancer limited to the mucosa or with slight submucosal invasion (< 1,000 μm below the muscularis mucosa; sm1) (*19*), 20 mm is the largest size that can be easily resected en bloc by polypectomy or snare EMR (*20*).

ii) *Criteria for additional treatment after ER of colorectal tumors*. Surgical resection with lymph node dissection is recommended when the vertical margin is positive and any of the findings in Figure 2 are observed in histological examination of the resected specimen (*2*).

iii) *Follow-up observation after ER of colorectal tumors*. Recurrence after endoscopic treatment for pT1 cancer is often within three years. When en bloc endoscopic resection is completed with a negative margin, annual endoscopic follow-up is recommended. Semi-annual surveillance with endoscopy is recommended with a positive horizontal margin (*21,22*). When an additional intestinal resection is not carried out for pT1 cancer, image diagnoses such as CT and tumor markers surveillance should be performed (*2*). In 2020, colonoscopic screening and surveillance guidelines were proposed by the Japanese Society of Gastroenterology. If there are no more than two adenomas, except for advanced adenoma, on the first total colonoscopy, follow-up after 3-5 years is required. If there are 3-9 adenomas except for advanced adenoma or advanced neoplasia follow-up after 3 years is recommended. If 10 or more non-advanced adenomas, Tis/pT1 cancer, or over 20 mm neoplasia are found and resected, follow-up after 1 year surveillance by TCS is recommended (*23*).

Outcomes of ER

Long-term outcomes after ER

Table 1 and Table 2 show recent reports list of complete resection rate, recurrence rate, underwent additional treatment rate and 5 years outcomes after ER for tumors of esophagus, stomach and colorectum. For esophageal cancer, 5-year cause specific survival rate for EP/LPM cancer, MM-SM1 and SM2 cancer were 98-100%, 93-100% and 100%, respectively (*24-28*). Besides, it was reported that the 5 years survival rate was 84.8% for complete resection, and 78.2% for incomplete resection (*29*). The long-term outcome after ER for MM/SM1 ESCC was favorable with additional prophylactic therapy (*25*). For gastric cancer, 5-year cause specific survival rate was reported as 99.9-100%, local recurrence rate was reported as 0.24-0.63% after ER (*30-33*). For colorectal cancer, 5-year cause specific survival rate was 98.6-100% (*24,34*). Komeda *et al.* reported that piecemeal resection was the only significant risk factor associated with local recurrence after ER (*35*). Although it has been reported, it has been considered that "death from metachronous cancer after ESD" has become a new issue in the ESD era, and it is necessary to raise awareness of the need for long-term (permanent) surveillance.

Complications

Major complications are bleeding and perforation. Table 3 shows the complication rate of ER for tumors of esophagus (*28,29,36*), stomach (*30-32*) and colorectum (*24,34,37*). Bleeding occurred in 0-1.2%, 4.2-6.3%, and 0-2.4%, respectively. Perforation occurred in 0-1.6%, 1.5-3.3%, and 0-6%, respectively. The incidence of post-

Table 1. complete resection rate, recurrence rate and underwent additional treatment rate of endscopic resection

site	lesions	complete resection rate(%)	local reccurence rate (%)	additiomal treatment (%)	Auther	*Ref.*
Esophagus	396	73.5	3.3	4.5	Oda *et al.* 2020	(*26*)
	1,070	93.6	-	-	Tachimori *et al.* 2019	(*29*)
Stomach	785	-	-	6.1	Mizumoto *et al.* 2018	(*27*)
	204	-	2.0	-	Nakagawa *et al.* 2014	(*36*)
	10,926	91.6	-	7.8	Suzuki *et al.* 2019	(*32*)
Colorectum	10,658	-	0.63	-	Tanabe *et al.* 2017	(*14*)
	1332	95.4	0.45	0.45	Nakamura *et al.* 2015	(*31*)
	421	92.9	0.24	0.24	Tanabe *et al.* 2014	(*30*)
	1,412	-	2.2	-	Komeda *et al.* 2019	(*35*)
	150	85.4	1.1	-	Kuwai *et al.*2017	(*34*)
	482	92.9	0.2	-	Takahashi *et al.* 2017	(*24*)
	150	97.3	-	-	Nawata *et al.* 2014	(*37*)

Abbreviations: -; No data

Table 2. outcomes of endoscopic resection

site	lesions	Depth	5-Year Overall Survival (%)	5-Year Cause Specific Survival (%)	Author	*Ref.*
Esophagus	396	HGN/EP/LPM/MM	95.1	99.1	Oda *et al.* 2020	(*26*)
	1,131	EP	84.7	-	Tachimori *et al.* 2019	(*29*)
		LPM/MM	86.4	-		
		SM1/SM2	74.5	-		
	102	MM/SM1	84.1*	97.5*	Takahashi *et al.* 2018	(*25*)
	60	HGN/EP/LPM	95	100	Nagami *et al.* 2017	(*28*)
	19	MM/SM1	84*	100*		
	4	SM2	75*	100*		
Stomach	1,537		92.6	99.9	Suzuki *et al.* 2016	(*33*)
	1,332		92.3	-	Nakamura *et al.* 2015	(*31*)
	421		-	100	Tanabe *et al.* 2014	(*30*)
Colon	150		94.1	98.6	Kuwai *et al.*2017	(*34*)
	482		-	100	Takahashi *et al.* 2017	(*24*)

Abbrebiations: HGN: High-grade neoplasia; EP: invasion to the depth of the epithelium; LPM: invasion to the depth of the lamina propria; MM: invasion to the depth of the muscularis mucosae; SM1: submucosal invasion to 200 μm or less; SM2: deep submucosal invasion exceeding 200 μm; *:patients took additinoal therapy was included; -: no data

Table 3. Complication rate of endoscopic resection

site	lesions	bleeding	perforation	Author	*Ref.*
Esophagus	1,207	0.1	1.6	Tachimori *et al.* 2019	(*29*)
	83	1.2	0	Nagami *et al.* 2017	(*28*)
	242	0	0.44	Nakagawa *et al.* 2014	(*36*)
Stomach	10,926	4.4	2.3	Suzuki *et al.* 2019	(*32*)
	1,332	4.2	1.5	Nakamura *et al.* 2015	(*31*)
	421	6.3	3.3	Tanabe *et al.* 2014	(*24*)
Colon	150	2.4	0	Kuwai *et al.*2017	(*34*)
	482	1.6	6	Takahashi *et al.* 2017	(*24*)
	150	0	0	Nawata *et al.* 2014	(*37*)

ER stenosis in esophageal cancer was 2.7-16.7% (*4,5,29*).

Future perspectives

In Japan, with the aging of the population, there are increasing opportunities to treat elderly patients aged 75 and over. Therefore, the safety and efficacy of ER in elderly patients are important. It was reported that the short or long-term outcomes of the elderly and the younger groups were comparable, although the rate of additional treatments was low in the elderly (*24,38*). Similarly, a trial on indications of endoscopic submucosal dissection for elderly patients with early gastric cancer (JCOG1902) has been conducted. Additional treatment might be too invasive for the elderly or patients with severe comorbidities. The risk of additional therapy should be considered against the risk of lymph node metastasis, considering the life expectancy of such patients.

In April 2014, laparoscopic and endoscopic cooperative surgery (LECS) was covered by insurance as a combination of endoscopic surgery and laparoscopic local gastrectomy. In this technique, full-thickness excision of the lesion using the ESD technique and

suturing the gastric wall defect with a laparoscope is performed. LECS for gastric cancer is contraindicated due to the possibility of intraperitoneal cancer cell dissemination and is currently mainly used for gastrointestinal stromal tumor (GIST) of the stomach (*39*). Recently, a combination of laparoscopic approaches to neoplasia with non-exposure technique (CLEAN-NET) and non-exposed endoscopic wall-inversion surgery (NEWS) has been devised and are expected to be minimally invasive treatments for patients with EGC (*40,41*).

Conclusion

Many endoscopic treatment technologies have been developed or improved in Japan, many of which are at the forefront of the world. In Japan, where endoscopic treatment has become the standard for early cancer and precancerous lesions, the quality of endoscopic treatment is high, and the development of more advanced and safe endoscopic technology is expected in the future. In an aging society, it is necessary to consider whether to provide additional treatment for the patient's life expectancy and risk of lymph node metastasis. In the future, it is desirable to develop a minimally invasive technique and apply additional resection according to the patient's background.

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MINI-REVIEW

Surgical strategies for treatment of clinical T4 esophageal cancer in Japan

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Abstract: Definitive chemoradiation (dCRT) is the mainstay treatment for cStage IVa esophageal squamous cell carcinoma (ESCC) with good performance status (PS), according to standard practice guidelines. Salvage surgery may incur operation complications and risk of mortality. According to the esophageal cancer practice guidelines outlined by the Japan Esophageal Society, when a tumor is residual and recurrent, chemotherapy and palliative symptomatic treatment is continued. However, salvage operation has been selected as a therapeutic option for recurrent or residual tumors after dCRT. There is weak evidence for not recommending surgery for cStage IVa ESCC exhibiting residual disease following dCRT. It has been reported that during salvage surgery the only prognostic factor that is thought to be performed is complete resection (R0), but at the same time, salvage esophagectomy increases the incidence of postoperative complications and mortality. The phase II chemoselection study by Yokota T *et al.* in Japan showed that multidisciplinary treatment initiated by induction therapy, in which docetaxel is added to cisplatin and 5-fluorouracil, resulted in a good prognosis in the short term. In this review, we discuss the surgical strategy and future of unresectable clinical T4 (cT4) ESCC.

***Keywords*:** esophageal squamous cell carcinoma, clinical T4 (cT4), definitive chemoradiation, salvage surgery, conversion surgery

Introduction

Esophageal cancer is one of the most malignant worldwide. In Western countries, adenocarcinoma is prevalent, however, in Eastern countries squamous cell carcinoma is more common than adenocarcinoma. Within the esophageal cancer practice guidelines outlined by the Japan Esophageal Society, assessment of the general condition and performance status (PS) of the patient is important for determining the treatment strategy for cStage IVa esophageal squamous cell carcinoma (ESCC). For patients exhibiting cStage IVa status with good PS, definitive chemoradiotherapy (dCRT) of greater than 50 Gy is used as a first mainstay and standard treatment to attempt curability (*1-3*). Figure 1 shows the algorithm for cStage IVa status in esophageal cancer practice guidelines as edited in 2017. When a tumor disappears following dCRT and exhibits complete response (CR), chemotherapy or follow-up is continued. On the other hand, when a tumor is residual and recurrent, chemotherapy and palliative symptomatic treatment remains ongoing.

However, salvage operations have been selected as a therapeutic option for recurrent or residual tumors after dCRT by esophageal surgeons. In Japanese practice guidelines, there is weak evidence to recommend salvage operation for cStage IVa ESCC exhibiting residual and recurrent disease after dCRT. When a local residual or recurrent tumor occurs after CRT intervention for ESCC, salvage surgery or endoscopic treatment may result in a better prognosis (*4-8*). It has been reported that during salvage surgery the only prognostic factor is thought to be correlated with complete resection (R0), however, salvage esophagectomy does increase incidence of postoperative complications and mortality.

In Western countries, palliative symptomatic therapy is the primary treatment for unresectable ESCC, and a salvage operation is rarely performed. The phase II chemoselection study by Yokota T *et al.* conducted in Japan entailed multidisciplinary treatment started by induction therapy (chemoselection), in which docetaxel was added to cisplatin and 5-fluorouracil (5-FU). The subsequent conversion surgery resulted in a good short-term prognosis (*9*). In contrast, the Kitasato digestive

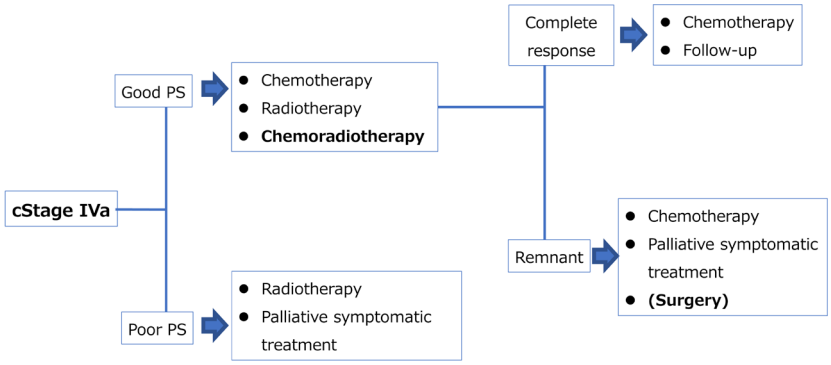

Figure 1. Esophageal cancer practice guidelines edited by the Japan Esophageal Society (JES), 2017. PS, performance status.

Table 1. Representative definitive CRT regimen for cT4 ESCC in Japan

Year	Trial name	Drugs	Regimen	RT dose (Gy)	CR rate (%)	Survival	*Ref.*
1999		CF	5-FU 400 mg/m^2, d1-5, 8-12, 29-33, 36-40, CDDP 75 mg/m^2, d1, 29	60	33	5 years: 14%	(*12*)
2004	JCOG9516	CF	5-FU 700 mg/m^2, d1-4, 29-32, CDDP 75 mg/m^2, d1, 29	60	15	2 years: 31.5%	(*13*)
2015	JCOG0303	CF	5-FU 700 mg/m^2, d1-4, 29-32, CDDP 70 mg/m^2, d1, 29	60	0	2 years: 25.9%	(*14*)
		CF	5-FU 200 mg/m^2, d1-5, 8-12, 15-19, 22-26, 29-33, CDDP 4 mg/m^2, d1, 29	60	1.4	2 years: 25.7%	(*14*)
2014	KDOG0501	DCF	5-FU 400 mg/m^2, d1-5, 15-19, 29-33, 43-47, CDDP 40 mg/m^2, d1, 15, 29, 43, DOC 20-40 mg/m^2, d1, 15, 29, 43	50.4-61.2	42.1	1 year: 63.2%	(*10,15*)

CRT: chemoradiation therapy; ESCC: esophageal squamous cell carcinoma; JCOG: Japan Clinical Oncology Group trial; KDOG: Kitasato Digestive Disease oncology Group trial; C: Cisplatin; F: 5-FU; D: Docetaxel.

disease oncology group (KDOG) 0501 phase I trial of docetaxel added to cisplatin and 5-fluorouracil (5-FU) in combination with radiotherapy resulted in a CR rate of 42.1% (*10*). A new study (Japan Clinical Oncology Group (JCOG) 1510: TRIANgLE) is currently ongoing (*11*). Although it remains a challenging surgery, performing aortic stent insertion and total pharyngo-laryngectomy with mediastinal tracheostomy is another option for intervention. In this review, we discuss surgical strategies and potential outcomes of unresectable clinical T4 (cT4) ESCC.

Definitive chemoradiation data for unresectable esophageal squamous cell carcinoma

In terms of recent esophageal cancer practice guidelines in Japan, the assessment of general condition and PS is important in making treatment decisions for cStage IVa ESCC. For patients exhibiting cStage IVa status with good PS, dCRT is the first mainstay treatment with an aim for curability (*1,2*). This treatment regimen is standard for unresectable cT4 ESCC. Table 1 lists several clinical trials including unresectable cT4 ESCC in Japan.

Ohtsu *et al.* reported the efficacy of dCRT consisting of concurrent 60 Gy radiotherapy using cisplatin and 5-fluorouracil (CF) for cT4 and/or M1 lymph node (LYM) with ESCC (*12*). The rate of complete response (CR) was 33% (18/54 patients) in a single-institution study. In 2004, the JCOG9516 study was reported as a phase II trial of dCRT for T4 and/or M1 LYM (*13*). The overall response rate was 68.3%, with a CR rate of 15%. Median survival time was 10 months and the 2-year survival rate was 31.5% in this multicenter trial. In 2015, another multicenter study (JCOG0303) was reported with a randomized phase II/III trial comparing standard CF versus a daily low-dose of CF with concurrent 60 Gy radiotherapy for treating cT4 unresectable regional lymph node metastasis (*14*). The median and 3-year overall survival rates of low-dose CF versus standard CF were 13.1 and 14.4 months, and 25.9% and 25.7%, respectively. There was no obvious advantage incurred using a low-dose over the standard CF regimen. In 2008 and 2014, another study (KDOG0501) reported on a phase I/II trial evaluating the safety and efficacy of dCRT employed a docetaxel with CF (DCF) regimen (DCF-R) for T4 and/or M1LYM ESCC. The total radiation dose was initially set at 61.2 Gy, but this was lowered to a multiple-field irradiation level of 50.4 Gy. The overall CR rate was 52.4%: 33.3% in the 61.2 Gy group and 60.0% in the 50.4 Gy group. The median overall survival was 29.0 months and the 3-year survival rate was 43.9%. However, a major toxicity of Grade 3 or more occurred frequently (*10,15*).

Salvage esophagectomy for local residual tumor after dCRT

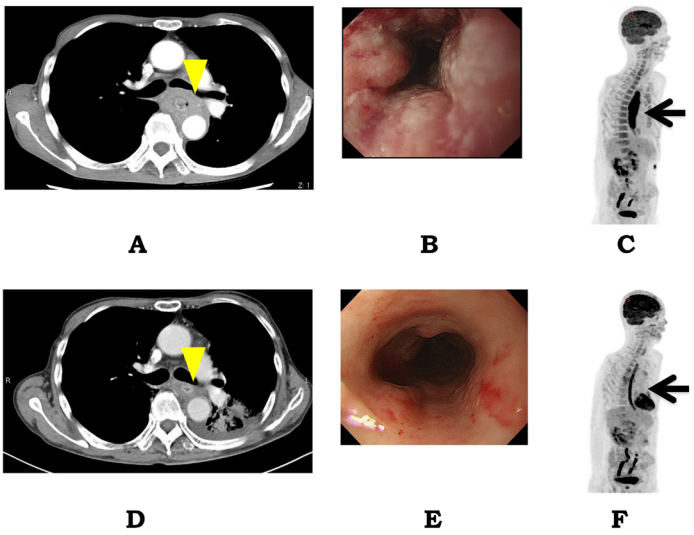

Figure 2. A case of clinical T4 esophageal squamous cell carcinoma (ESCC) in pre-treatment status (A-C) and status after definitive chemoradiation therapy (dCRT) (D-F). (A) CT imaging of left bronchial level; **(B)** Endoscopic film of esophageal cancer; **(C)** ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET) scan of esophageal cancer; **(D)** CT imaging of left bronchial level; **(E)** Endoscopic film of esophageal cancer; **(F)** FDG-PET scan after dCRT.

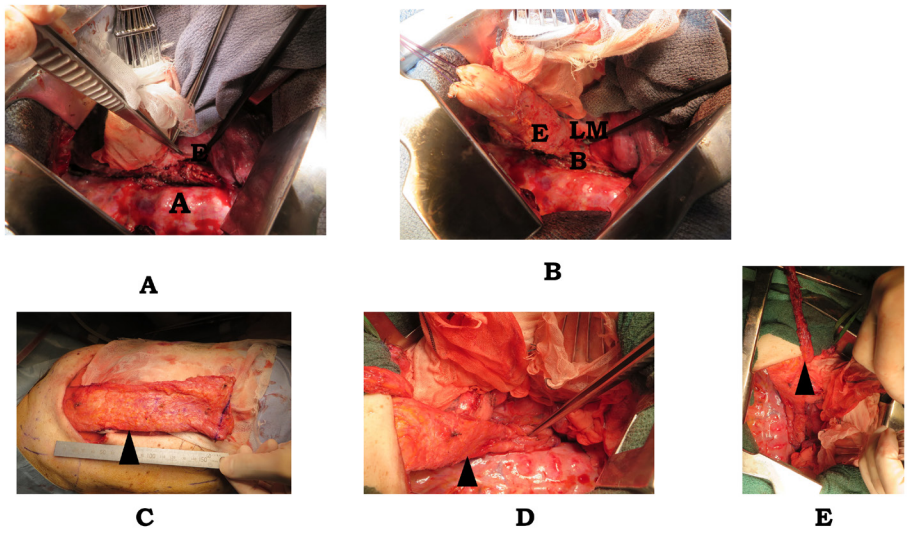

Figure 3. Intraoperative findings of the same case wherein salvage esophagectomy with open thoracotomy was performed. Severe adhesion and fibrosis was observed, and the membrane between the esophagus and adjacent organs was unrecognizable **(A)**. View after esophagectomy is shown in **(B)**. A, Adhesion; E, Esophagus; LMB, left main bronchus. View of filling material available in muscles is shown in **(C, D)** latissimus dorsi and **(E)** intercostal muscle.

In general, salvage surgery for local residual or recurrent tumors occurring after dCRT may increase the difficulty of operation and risk of mortality (*1-8*). However, this intervention is currently the only treatment leading to survival following dCRT. If dCRT is capable of R0 resection following induction CRT with 30-40 Gy irradiation using CF, we could perform esophagectomy, if required. However, deciding on the capability of surgical indications after induction CRT for cT4 ESCC is difficult. Furthermore, the recently introduced dCRT technique may improve outcomes more than induction CRT (*16*).

Figure 2 and Figure 3 describe a clinical case of salvage operation after dCRT. At first diagnosis the patient's clinical stage was cT4b (left main bronchus, aorta) N2M0 and cStage IVa status. First, the subject underwent dCRT using CF with a 60 Gy irradiation dose. After dCRT, the patient underwent two courses of chemotherapy with CF. Subsequently the residual tumor was of the thoracic esophagus, and ESCC was decreased (cT4bN2M0→ycT2N0M0). We performed salvage esophagectomy and with open thoracotomy and laparotomy reconstructed by stomach roll. During the intraoperative findings, there was severe adhesion

and fibrosis present, and the membrane between the esophagus and adjacent organs was unrecognizable (Figure 3A and 3B).

In Japan, the historical surgical treatment for unresectable cT4 ESCC has also changed over time from preoperative radiotherapy, combined resection of the adjacent organs with esophagectomy, and to dCRT with salvage surgery (*6*). However, preoperative radiation therapy and combined resection of the adjacent organs were not shown to be significant. At present, esophageal surgeons routinely perform salvage operation for removal of residual and remnant ESCC after dCRT, and there exists weak evidence within Japanese guidelines for Stage IVa ESCC due to surgery efficacy along with a high risk for mortality and morbidity.

Most surgeons have regarded adjacent organ invasion as indicative of poor prognosis and associated with high postoperative mortality and morbidity. There are two primary limitations of salvage esophagectomy for cT4 ESCC. First, the main prognostic factor is thought to be complete resection (R0). Intraoperative macroscopic inspection after dCRT is often unrecognizable. Secondly, preoperative assessment (*e.g.*, imaging findings) entail limitations for predicting pathologic T4. The accuracy rates for detection of pathologic T4 and curability prediction following operation are insufficient. Only patients with definitive evidence of unresectability (R2) should be excluded from esophagectomy (*8*).

^{18}F-fluorodeoxyglucose-Positron emission tomography (FDG-PET) has been covered in national health insurance for esophageal cancer. In surgically resectable ESCC, the value of standardized uptake value (SUV) max of FDG-PET is effective for responders of neoadjuvant therapy. Recently, PET has proven to be an important modality for evaluation of treatment for T4 ESCC (*17*). Conversely, Jingu *et al.* reported that FDG -PET may not contribute to improving survival for locally advanced unresectable ESCC (*18*).

Many studies have reported about salvage esophagectomy for unresectable cT4 ESCC in Japan (*19-34*). Ikeda *et al.* reported on 13 of 37 patients after dCRT who underwent salvage surgery. R0 resection was performed in 12 patients (*27*). Another study protocol (JCOG0303) described chemoradiotherapy using CF with 60 Gy radiation for cT4 unresectable ESCC. Of the 71 patients who received dCRT, 12 cases (17%) underwent salvage surgery for residual or recurrent tumors. The median overall survival was 13 months, and the 1 or 3-year survival rates were 56.8% and 27.6%, respectively. The prognoses of R1 and R2 patients were unfavorable (*14*). Ohkura *et al.* reported methods for lymph node dissection of salvage esophagectomy. If possible, salvage esophagectomy was performed with typical lymph node dissection, including prophylactic dissection, which led to improved prognosis (*28*). Pimiento *et al.* reported that historical incomplete resections and poor survival rates often result in surgery being palliative rather than curative. We have demonstrated that neoadjuvant therapy and downstaging of T4 tumors leads to increased R0 resections and improvements in overall and disease-free survival (*21*).

During salvage operations, the structure surrounding the esophagus and adjacent organs were unrecognizable during salvage operations for cT4 ESCC. These tissues bled easily and were fragile. In addition, it was impossible to distinguish cancer from severe fibrosis. Pathological diagnosis of intraoperative frozen sections at marginal lesions were sometimes diagnostically effective. The tracheal membranous portion was easily torn and damaged. If the boundary between the trachea and esophagus was undecipherable, the esophageal surgeon should have a filling material available (*e.g.*, latissimus dorsi or intercostal muscles) (Figure 3C and 3E). Surgeons should always be fully aware of when to return to the operation.

Consensus of conversion surgery for unresectable ESCC

Recently, induction chemotherapy is becoming available for intervention for unresectable cT4 ESCC. DCF induction chemotherapy for T4 ESCC reduced esophageal perforation and increased resectability and increased survival compared to CRT alone. Therefore, DCF induction chemotherapy may be an effective option for initial induction treatment of T4 ESCC (*35-39*). DCF and subsequent esophagectomy achieved R0 resection in 50% of patients and was associated with better long-term oncological outcomes for unresectable ESCC with acceptable systemic status (*35*). The available induction DCF chemotherapy for unresectable ESCC was considered from a trial of head and neck cancer. This strategy is known as conversion therapy and the related surgical intervention is termed conversion surgery. The clinical significance of conversion surgery for ESCC remains unknown. Yokota *et al.* reported on a multicenter phase II trial in which the safety and efficacy of chemo-selection using induction chemotherapy with DCF for unresectable ESCC was assessed (*9*). Treatment was initiated with induction DCF, followed by conversion surgery for resectable tumors, or by CRT if tumors were unresectable. Twenty of the 48 patients enrolled (41.7%) underwent conversion surgery and R0 resection was achieved in 39.6%. The one-year overall survival rate had an improved prognosis of 67.9%. These results suggest that chemo-selection with DCF induction chemotherapy followed by conversion surgery is a promising strategy for unresectable ESCC. At present, an ongoing trial is in phase III (JCOG1510) and compares two therapies: DCF induction versus dCRT plus salvage surgery (*11*).

Recently, challenging salvage operations were performed using thoracic endovascular aortic repair (TEVER) and salvage pharyngo-laryngectomy with mediastinal tracheostomy. TEVAR has been used for

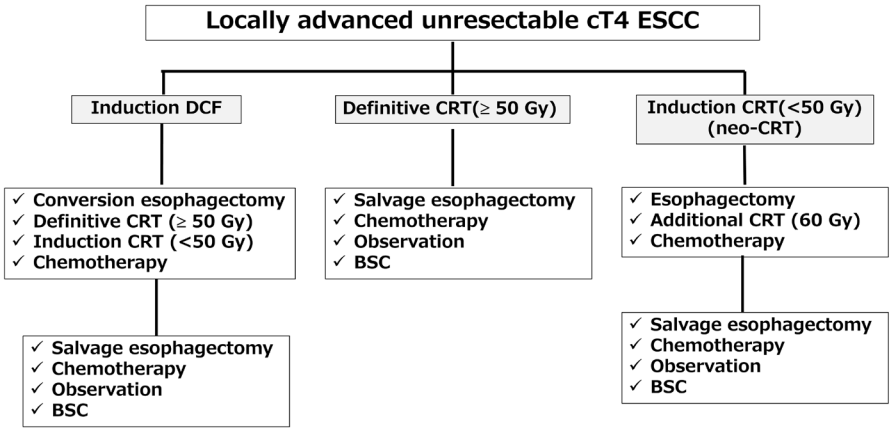

Figure 4. Surgical strategies for cT4 ESCC in Japan.

esophageal cancer invading the aorta to avoid esophago-aortic fistula. Nakajima *et al.* performed salvage esophagectomy combined with partial resection of the aortic wall after TEVAR in four patients following CRT, and acceptable short-term outcomes were obtained (*40,41*).

Conclusion

In conclusion, we summarized the treatment algorism for unresectable cT4 ESCC (Figure 4). In the past, the prognosis for cT4 ESCC was poor because of limited therapy; however, induction chemotherapy (chemoselection) or DCF in combination with radiation therapy may result in better outcomes.

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Current status of immune checkpoint inhibitor therapy for advanced esophageal squamous cell carcinoma

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Abstract: Esophageal cancer is the seventh most common cancer, with an estimated 572,000 new cases, and the sixth most common cause of cancer-related deaths in 2018 with 509,000 annual worldwide deaths. Advanced esophageal squamous cell carcinoma (ESCC) is one of devastating tumors with a 5-year survival rate of less than 5% in patients with metastatic disease. Treatment options for patients with advanced ESCC are limited. Current guidelines recommend chemotherapy containing a platinum and a fluoropyrimidine agent as a first-line treatment. Recently, immune checkpoint inhibitors (ICIs), including nivolumab and pembrolizumab, have demonstrated antitumor activity and clinical efficacy in patients with advanced ESCC that is refractory or intolerant to first-line chemotherapy. ICIs are game-changers that not only transformed oncological strategy but also have a wide range of clinical potential in combination with conventional cytotoxic chemotherapy and radiotherapy. There is still an urgent, unmet need for reliable treatment options to conquer this intractable disease.

Keywords: esophageal squamous cell carcinoma, immune checkpoint inhibitor, nivolumab, pembrolizumab

Introduction

Esophageal cancer is the seventh most common cancer, with an estimated 572,000 new cases, and the sixth most common cause of cancer-related deaths in 2018, with 509,000 deaths annually worldwide (*1*). Esophageal cancer comprises squamous cell carcinoma (SCC), adenocarcinoma (AC), and other minor histological subtypes. SCC and AC have distinct etiological and clinicopathological features.

Esophageal SCC (ESCC) is generally in decline in North America and Europe, where alcohol drinking and smoking are the major risk factors. However, the incidence is still extremely high in parts of Asia and Sub-Saharan Africa. In contrast, the incidence of esophageal AC (EAC) is rapidly rising around high-income countries in North America and Western Europe, where increasing obesity and gastroesophageal reflux disease may explain the high prevalence (*1,2*).

Large-scale genomic analysis of ESCC using next-generation sequencing revealed distinct profiles of genomic alterations between ESCC and EAC. KRAS and ERBB2 were more frequently altered in EAC than in ESCC, whereas NOTCH1 was more commonly altered in ESCC than in EAC (*3*). Molecularly targeted therapies focusing on HER2 (trastuzumab) or vascular endothelial growth factor (ramucirumab) are applicable in patients with gastroesophageal AC (*4-6*). The number of non-synonymous somatic mutations in ESCC was relatively high among solid tumors except for colorectal cancer. However, the diver mutation has not been identified in ESCC (*7,8*).

A multidisciplinary approach involving surgery, radiotherapy, and chemotherapy is the mainstay of treatment that improves prognosis in resectable ECC. However, the 5-year survival rate is less than 5% in patients with unresectable advanced ECC, which is defined as locally advanced, metastatic, or recurrent ECC without indication for curative surgery (*9*). Although survival benefit over the best supportive care is not sufficiently demonstrated, fluoropyrimidine and platinum doublet chemotherapy is recommended as an acceptable first-line therapy for patients with metastatic or recurrent ESCC according to current guidelines (*10-12*). In Japan, combination chemotherapy of cisplatin and 5-fluorouracil (CF) is recognized as the first-line regimen based on two phase 2 trials (JCOG8807 and JCOG9407) which demonstrated a response rate of 33.3-35.9% and median overall survival (OS) of 6.7 months (*13,14*). The clinical efficacy of docetaxel, cisplatin and 5- fluorouracil (DCF) regimen for advanced ESCC was demonstrated with a favorable response rate of around 60% in early phase trials (*15-17*). The incidence of grade 3/4 neutropenia and grade 3/4 febrile neutropenia were high as 43.6-90.0% and 10.0-22.2%, respectively. A phase 3 trial (JCOG 1314) comparing modified DCF with CF as

first-line chemotherapy in patients with metastatic or recurrent esophageal cancer is ongoing.

Taxanes (docetaxel and paclitaxel) and irinotecan are commonly used agents as second-line treatment in patients with advanced ESCC if the first-line therapy fails. Docetaxel as a single agent for metastatic esophageal cancer was effective with a response rate of 16% and median OS of 8.1 months, although grade 3/4 neutropenia occurred in 88% of patients (*18*). Weekly paclitaxel as second-line therapy for advanced or recurrent esophageal had shown efficacy with a response rate of 44.2% and median OS of 10.4 months. The most common grade 3/4 adverse events were neutropenia which occurred in 52.8%, and adverse events leading to discontinuation of therapy occurred in 34% of patients (*19*). Irinotecan administered in repeated 6-week cycles for patients with unresectable esophageal carcinoma demonstrated a response rate of 22.2% and median OS of 7.5 months, while grade 3/4 neutropenia and diarrhea occurred in 5% and 5.8% of patients, respectively (*20*). Paclitaxel, irinotecan, or oxaliplatin-based combination regimens have also been investigated as first or second-line therapy for advanced ESCC. The combination regimens showed some efficacy; however, a relatively high incidence of toxicities is of concern given the vulnerable conditions of the patients with advanced ESCC.

Immune checkpoint inhibitors (ICIs)

Immune checkpoints are a part of the immune system that modulates the immune response to attack tumor cells. Immune checkpoint molecules are present on T cells, antigen-presenting cells (APCs), and tumor cells. The interactions among the molecules stimulate either inhibitory or activating immune signaling pathways. The inhibitory signaling pathway plays a pivotal role in inducing immune tolerance and suppressing autoimmune responses. Some cancers escape from the attack by stimulating inhibitory signals. Immune checkpoint molecules include programmed cell death protein 1 (PD-1) and CTLA-4 (cytotoxic T-lymphocyte associated antigen 4) on T-cells and programmed death-ligand 1 (PD-L1) and B7-1/B7-2 on APCs, including tumor cells. Blocking the binding of PD-L1 to PD-1 or B7-1/B7-2 to CTLA-4 with an ICI allows the T-cells to activate and destroy the tumor cells (*21,22*). Immune checkpoint molecules and representative ICIs targeting inhibitory immunoreceptors are illustrated in Figure 1.

The clinical use of checkpoint inhibitors has dramatically changed the therapeutic strategy for malignant melanoma. The antitumor activity of these agents has been reported in gastrointestinal AC and SCC of the esophagus, head and neck, lung, and anus with promising efficacy (*23-28*). The first ICI approved by the Food and Drug Administration (FDA) was ipilimumab (anti-CTLA-4 antibody), followed by nivolumab and pembrolizumab (anti-PD-1 antibodies).

Tumor PD-L1 expression is thought to be correlated with tumor susceptibility to immune checkpoint inhibition and favorable prognosis. In patients with ESCC, the PD-L1 expression rate ranges from 15% to 83% in tumor cells, and from 13% to 31% in immune cells (*29-34*).

ICIs can induce unique side effects known as immune-related adverse events (irAEs). These irAEs are distinct from AEs of traditional cytotoxic agents and typically exhibit a delayed onset and prolonged duration. These irAEs are rare and generally low grade; however, some irAEs can be severe and may lead to fatal consequences. Although irAEs can affect almost any organ, frequent toxicities involve the skin, thyroid gland, lung, liver, and pituitary gland. The treatment includes temporary discontinuation of ICIs and the use of glucocorticoids as well as monoclonal antibody therapy or plasma exchange for severe cases. Monitoring the functions of some organs, such as the thyroid and liver, is recommended to detect irAE early before it becomes evident (*35*).

Nivolumab

Nivolumab is a human IgG4 monoclonal anti-PD-1 antibody that was approved for the treatment of melanoma by the FDA in 2014. Clinical trials revealed promising clinical efficacy with manageable adverse effects in several solid tumors, including advanced ESCC.

An open-label, multicenter, phase 2 trial (ONO-4538-07) investigated the safety and activity of nivolumab

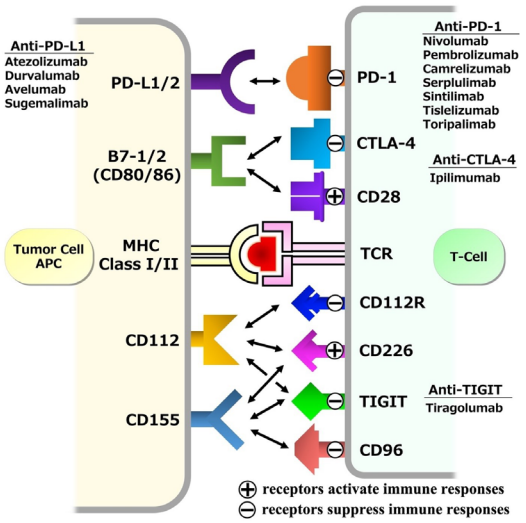

Figure 1. Immune checkpoint molecules and representative immune checkpoint inhibitor agents targeting inhibitory immunoreceptors. APC, antigen-presenting cell; CTLA-4, cytotoxic T-lymphocyte associated antigen 4; MHC, major histocompatibility complex; PD-1, programmed cell death protein 1; PD-L1/2, programmed cell death protein ligand 1 and 2; TCR, T-cell receptor; TIGIT, T-cell immunoreceptor with Ig and ITIM domains.

in patients with advanced ESCC that was refractory or intolerant to fluoropyrimidine-based, platinum-based, and taxane-based chemotherapies. Seventeen percent of 64 patients had an objective response with central assessment. The median OS and progression-free survival (PFS) were 10.8 months and 1.6 months, respectively. Common adverse events included pneumonia and interstitial lung disease, all of which were manageable with treatment discontinuation or appropriate care (*28*).

The randomized, open-label, phase 3 ATTRACTION-3 trial compared the clinical efficacy of nivolumab with that of chemotherapy in patients with unresectable advanced or recurrent ESCC that was refractory or intolerant to one previous line of fluoropyrimidine-based and platinum-based chemotherapy. The OS was significantly improved in the nivolumab group compared with the chemotherapy group (median 10.9 months *vs.* 8.4 months; HR for death 0.77, 95% CI 0.62-0.96, $p = 0.019$). PD-L1 expression in tumors was not correlated with the survival benefit with nivolumab, although patients with at least 1% PD-L1 expression had a 15% reduction in the risk of death than those with less than 1% PD-L1 expression. Despite the favorable OS in the nivolumab group, treatment with nivolumab did not provide a significant benefit in PFS over chemotherapy (median 1.7 months *vs.* 3.4 months; HR for progression or death 1.08, 95% CI 0.87-1.34). Common treatment-related adverse events included rash (11%), diarrhea (12%), and decreased appetite (11%) in the nivolumab group, whereas alopecia (47%), neutropenia (37%), and leukocytopenia (35%) were observed in the chemotherapy group. Serious adverse events and treatment-related deaths occurred in 16% of nivolumab patients and 23% of chemotherapy patients. Treatment-related deaths were reported in 1% of patients treated with both nivolumab and chemotherapy (*36*).

Pembrolizumab

Pembrolizumab is a selective human IgG4 monoclonal antibody designed to inhibit the interaction between PD-1 and its ligands, PD-L1 and PD-L2. The FDA approved pembrolizumab for clinical use in recurrent or metastatic head and neck SCC in June 2018, based on the KEYNOTE-012 study, a phase 1B trial that evaluated the safety and antitumoral activity of pembrolizumab in patients with head and neck cancer with any level of PD-L1 expression. The proportion of patients with an overall response was 18% (8 of 45 patients; 95% CI 8-32). Twenty-three out of 28 patients had maintained tumor shrinkage over six months (*37*).

KEYNOTE-028, a phase IB study, investigated the safety and antitumor activity of pembrolizumab in patients with PD-L1 positive, advanced solid tumors. Twenty-three patients with PD-L1 positive esophageal or gastroesophageal junction carcinoma received pembrolizumab after standard therapy failed. Overall responses were observed in 29.4% of patients with SCC and 40.0% of patients with AC. Twelve patients (52%) showed tumor shrinkage from baseline in target lesion burden. Median overall survival was 7.0 months, and the 12-month overall survival rates were 40% (*38*).

The phase 2 KEYNOTE-180 study evaluated the efficacy and safety of pembrolizumab for patients with advanced ESCC, EAC, or esophagogastric junction AC after two or more lines of systemic therapy. The response rate was 9.9% among 63 patients with ESCC, 13.8% among 58 patients with PD-L1-positive tumors, and 6.3% among 63 patients with PD-L1-negative tumors. The trial confirmed durable antitumor activity and manageable safety (*39*).

The randomized, open-label, global, phase 3 study, KEYNOTE-181, enrolled 628 patients with metastatic, locally advanced unresectable ESCC, EAC, or esophagogastric junction AC that progressed after one prior line of standard therapy (*40*). The patients were randomly assigned 1:1 to pembrolizumab or the investigator's choice of standard-of-care chemotherapy with paclitaxel, docetaxel, or irinotecan. A PD-L1-positive tumor was defined as the combined positive score (CPS) of 10 or more, as previously described in phase 2 KEYNOTE-180 study. Pembrolizumab provided a significantly better OS compared with chemotherapy in patients with metastatic ESCC and PD-L1 CPS $\geq$ 10 (Table 1). On retrospective stratified analysis, OS was remarkably improved in the pembrolizumab group compared with the chemotherapy group (median 10.3 months *vs.* 6.7 months; HR for death 0.64, 95% CI 0.46-0.90) among SCC patients with PD-L1 CPS $\geq$ 10. In patients with PD-L1 CPS $\geq$ 10, PFS was also improved in the pembrolizumab group compared with the chemotherapy group (median

Table 1. Results of the stratified analysis in the randomized phase 3 KEYNOTE-181 study

	Treatment	Patients	Median OS [95% CI] (months)	HR	*p* value
All	Pembrolizumab	314	7.1 [6.2-8.1]	0.89 [0.75-1.05]	0.08431
	Chemotherapy	314	7.1 [6.3-8.0]		
CPS $\geq$ 10	Pembrolizumab	107	9.3 [6.6-12.5]	0.70 [0.52-0.94]	0.00855
	Chemotherapy	115	6.7 [5.1-8.2]		
SCC	Pembrolizumab	198	8.2 [6.7-10.3]	0.77 [0.63-0.96]	0.00894
	Chemotherapy	203	7.1 [6.1-8.2]		

CI, confidence interval; CPS, combined positive score; HR, hazard ratio; OS, overall survival; SCC, squamous cell carcinoma.

2.6 months *vs.* 3.0 months; HR 0.73, 95% CI 0.54-0.97). Treatment-related adverse events were observed in 64% of patients in the pembrolizumab group and 86% of patients in the chemotherapy group. The common adverse events in the pembrolizumab group included fatigue (11.8%), hypothyroidism (10.5%), decreased appetite (8.6%), asthenia (7.0%), and nausea (7.0%). Immune-mediated adverse events and infusion reactions occurred in 23.2% of patients, comparable with results observed in previous studies (*28,36,39*).

Based on the results of the reliable phase 3 clinical trials, the Japan Esophageal Society issued a public statement recommending treatment with nivolumab as second-line therapy in patients with metastatic, locally advanced unresectable, and recurrent esophageal cancer irrespective of the PD-L1 expression status, and treatment with pembrolizumab as second-line therapy in patients with metastatic, locally advanced unresectable ESCC with PD-L1 CPS ≥ 10. Results from the pivotal clinical trials that demonstrated the antitumor activity and clinical efficacy of nivolumab and pembrolizumab are summarized (Table 2).

Camrelizumab is another humanized, selective IgG4 monoclonal antibody against PD-1. The antitumoral activity was proved across a variety of solid tumors (*41,42*). A randomized, open-label, phase 3 study, ESCORT trial was conducted in 43 hospitals in China. Six hundred seven patients with advanced, recurrent, or metastatic ESCC were randomly assigned to camrelizumab or chemotherapy (docetaxel or irinotecan) as second-line treatment. The result showed a favorable median OS of 8.3 months (95% CI 6.8-9.7) in the camrelizumab group, as compared to 6.2 months (5.7-6.9) in the chemotherapy group (hazard ratio 0.71 [95% CI 0.57-0.87], $p = 0.001$) (*43*).

Combination of immune checkpoint inhibitors and radiation therapy or cytotoxic agents

The standard treatment for unresectable locally advanced ESCC is chemoradiotherapy (CRT) (*44*). The potential synergy of combination therapy with ICIs and radiation is attracting attention lately. The abscopal effect signifies the regression of non-irradiated lesions located distally from the primary site of irradiation. A persuasive theory explains a systemic antitumor immune response may mediate the effect (*45,46*). The mechanism of the effect may include the recruitment and distribution to the microenvironment of T-cells, cytokine release, and enhanced presentation of tumor antigen. Preclinical studies indicated increased PD-L1 expression in irradiated tumors, which prompts the need for enhanced antitumor activity by combined therapy with anti-PD-1 antibodies and radiation in locally advanced ESCC (*47*). Furthermore, radiation can potentially promote the antigen presentation of tumors. This is one of the rationales that support the validity of combination therapy of radiation and ICIs (*48*).

A phase 3 PACIFIC trial compared durvalumab (anti-PD-L1 antibody agent) following platinum-based CRT with placebo following CRT for unresectable locally advanced non-small-cell lung cancer. Twelve months

Table 2. Results from a selected clinical trial using immune checkpoint inhibitors in advanced esophageal squamous cell carcinoma

Agents (Trial)	Phase	Line	Location	Histology	Enrolled Patients	Regimen	Response Rate	Median PFS	Median OS
Nivolumab (ONO-4583-07)	2	≥ 2	E	SCC	65	Nivolumab	17%	1.6M	10.8M
Nivolumab (ATTRACTION-3)	3	≥ 2	E/EGJ	SCC	419	Nivolumab *vs.* PTX or DTX	19% *vs.* 22%	1.7M *vs.* 3.4M	10.9M *vs.* 8.4M
Pembrolizumab (KEYNOTE-028)	1	≥ 2	E/EGJ	SCC/AC (PD-L1+)	23	Pembrolizumab	All: 30% SCC: 28% AC: 40%	1.8M	7.0M
Pembrolizumab (KEYNOTE-180)	2	≥ 3	E/EGJ	SCC/AC	121	Pembrolizumab	All: 9.9% SCC: 14.3% AC: 5.2% PD-L1(+): 13.8% PD-L1(-): 6.3%	All: 2.0M SCC: 2.1M AC: 1.9M PD-L1(+): 2.0M PD-L1(-): 2.0M	All: 5.8M SCC: 6.8M AC: 3.9M PD-L1(+): 6.3M PD-L1(-): 5.4M
Pembrolizumab (KEYNOTE-181)	3	2	E/EGJ	SCC/AC	628	Pembrolizumab *vs.* PTX or DTX or CPT-11	All: 13.1% *vs* 6.7% PD-L1(+): 21.5% *vs.* 6.1% SCC: 16.7% *vs.* 7.4%	All: 2.1M *vs.* 3.4M PD-L1(+): 2.6M *vs.* 3.0M SCC: 2.2M *vs.* 3.1M	All: 7.1M *vs.* 7.1M PD-L1(+): 9.3M *vs.* 6.7M SCC: 8.2M *vs.* 7.1M
Camrelizumab (ESCORT)	3	2	E	SCC	457	Camrelizumab *vs.* DTX or CPT-11	20.2% *vs.* 6.4%	1.9M *vs.* 1.9M	8.3M *vs.* 6.2M

AC, adenocarcinoma; CPS, combined positive score; CPT-11, irinotecan; DTX. docetaxel; E, esophagus; EGJ, esophagogastric junction; HR, hazard ratio; M, months; OS, overall survival; PD-L1, programmed cell death protein ligand 1; PFS, progression-free survival; PTX, paclitaxel; SCC: squamous cell carcinoma.

of durvalumab after CRT significantly prolonged both PFS (16.8 *vs.* 5.6 months; HR: 0.52; 95% CI: 0.42-0.65, $p < 0.001$) and OS (not reached *vs.* 28.7 months, HR: 0.68; 99.73% CI: 0.47-0.997; $p = 0.0025$) compared with acceptable radiation or ICI-induced pneumonitis (*49*).

Phase 3 clinical trials investigating the efficacy and safety of the combination of ICIs with chemoradiation therapy for locally advanced ESCC are in progress (NCT04543617, NCT04550260).

A phase 3 randomized, international, double-blind study KEYNOTE-590 (NCT03189719) was conducted to compare pembrolizumab plus chemotherapy (CF regimen) with chemotherapy alone in patients with locally advanced, unresectable, or metastatic EAC, ESCC, or Siewert type 1 gastroesophageal junction adenocarcinoma. 749 patients were enrolled, and the OS superiority of pembrolizumab plus chemotherapy was demonstrated in patients with ESCC (median 12.6 *vs.* 9.8 months; HR 0.72; 95% CI, 0.60-0.88; $p = 0.0006$), CPS ≥ 10 (median 13.5 *vs.* 9.4 months; HR 0.62; 95% CI, 0.49-0.78; $p < 0.0001$), and all patients (median 12.4 *vs.* 9.8 months; HR, 0.73; 95% CI, 0.62-0.86; $p < 0.0001$). Addition of pembrolizumab also provided longer PFS (median 6.3 *vs.* 5.8 months; HR 0.65; 95% CI, 0.55-0.76; $p < 0.0001$) and better response rate (45.0% *vs.* 29.3%; $p < 0.0001$), with acceptable safety profile (*50*). Based on the result, the FDA has approved pembrolizumab for use in combination with platinum and fluoropyrimidine-based chemotherapy for patients with metastatic or locally advanced esophageal or gastroesophageal carcinoma in March 2021.

Dual immune checkpoint inhibition therapy to enhance the efficacy of immunotherapy has also been investigated. The CheckMate 648 (NCT03143153) study is a randomized phase 3 trial to compare the efficacy of nivolumab plus CF *vs.* nivolumab plus ipilimumab *vs.* CF alone as first-line therapy in patients with unresectable advanced, recurrent, or metastatic ESCC. Both nivolumab plus chemotherapy and nivolumab plus ipilimumab demonstrated superior OS over chemotherapy (median 15.4 *vs.* 13.7 *vs.* 9.1 months) in patients with tumor cell PD-L1 ≥ 1%. The OS superiority

Table 3. List of ongoing randomized phase 3 clinical trials for advanced esophageal cancer

NCT number (Title of the trial)	Target	Agent	Line	Location	Histology	Eligible Condition	Treatment Arm	Enrolled Patients	Primary Outcome
NCT04543617 (SKYSCRAPER 07)	PD-L1 TIGIT	Atezolizumab Tiragolumab	1	E	SCC	LA	Tiragolumab + Atezolizumab + dCRT placebo + Atezolizumab + dCRT placebo + dCRT	750	OS/PFS
NCT04540211 (SKYSCRAPER 08)	PD-L1 TIGIT	Atezolizumab Tiragolumab	1	E	SCC/AC	LA, M, R	Atezolizumab + Tiragolumab + PC placebo + PC	450	OS/PFS
NCT04426955	PD-1	Camrelizumab	1	E	SCC	LA	Camrelizumab + PC + RT placebo + PC + RT	390	PFS
NCT03691090	PD-1	Camrelizumab	1	E	SCC/AC	LA, M, R	Camrelizumab + PC placebo + PC	548	OS/PFS
NCT04550260	PD-L1	Durvalumab	1	E	SCC	LA	Durvalumab + dCRT placebo + dCRT	600	PFS
NCT04210115 (KEYNOTE 975)	PD-1	Pembrolizumab	1	E/GEJ	SCC/AC	LA	Pembrolizumab + FP or FOLFOX + RT placebo + FP or FOLFOX + RT	600	OS/EFS
NCT03189719 (KEYNOTE 590)	PD-1	Pembrolizumab	1	E/GEJ	SCC/AC	LA, M	Pembrolizumab + FP placebo + FP	749	OS/PFS
NCT03958890	PD-1	Serplulimab	1	E	SCC	LA, M, R	Serplulimab + FP placebo + FP	489	PFS
NCT03748134 (ORIENT 15)	PD-1	Sintilimab	1	E	SCC	LA, M, R	Sintilimab + FP or PC placebo + FP or PC	676	OS
NCT04187352	PD-L1	Sugemalimab	1	E	SCC	LA, M, R	Sugemalimab + FP placebo + FP	420	OS/PFS
NCT03957590	PD-1	Tislelizumab	1	E	SCC	LA	Tislelizumab + PC + RT placebo + PC + RT	316	PFS
NCT03783442	PD-1	Tislelizumab	1	E	SCC	LA, M, R	Tislelizumab + chemotherapy placebo + chemotherapy	649	OS/PFS
NCT03829969 (JUPITER 06)	PD-1	Toripalimab	1	E	SCC	LA, M, R	Toripalimab + PC placebo + PC	500	OS/PFS
NCT03143153 (CheckMate 648)	PD-1 CTLA-4	Nivolumab Ipilimumab	1	E	SCC/AC	LA, M, R	Nivolumab + Ipilimumab Nivolumab + FP FP	970	OS/PFS
NCT03430843	PD-1	Tislelizumab	2	E	SCC	LA, M	Tislelizumab Paclitaxel or Docetaxel or Irinotecan	513	OS

AC, adenocarcinoma; Adj, adjuvant chemotherapy; CTLA-4, cytotoxic T-lymphocyte associated antigen 4; dCRT, definitive chemoradiotherapy; DFS, disease-free survival; E, esophagus; EFS, event-free survival; FP, 5-fluorouracil + cisplatin; GEJ, gastroesophageal junction; LA, locally advanced disease; M, metastatic disease; OS, overall survival; PC, paclitaxel and cisplatin; PD-1, programmed cell death protein 1; PD-L1, programmed cell death protein ligand 1; PFS, progression-free survival; R, recurrent disease; RT, radiotherapy; SCC, squamous cell carcinoma; TIGIT, T cell immunoreceptor with Ig and ITIM domains

was also confirmed in all patients with both nivolumab plus chemotherapy and nivolumab plus ipilimumab compared with chemotherapy alone: 13.2, 12.8, and 10.7 months, respectively (*51*).

Many phase 3 trials evaluating the efficacy of a wide range of combinations of ICIs with cytotoxic agents and radiation therapy are now in progress worldwide (Table 3). The results of these studies will dramatically change the practical strategy for the treatment of advanced ESCC furthermore.

Conclusion

Based on the results of randomized, phase 3 trials, nivolumab and pembrolizumab were approved by the Japanese Ministry of Health, Labor and Welfare as second-line therapy for patients with unresectable advanced esophageal cancer in February and August 2020, respectively. The approval of novel agents against esophageal cancer was granted for the first time in eight years in Japan. ICIs transformed oncological strategy and have a wide range of clinical potential in combination with conventional cytotoxic chemotherapy and radiotherapy.

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The primary tumor location in colorectal cancer: A focused review on its impact on surgical management

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Abstract: The primary tumor location (PTL) has attracted increasing attention in recent years for colorectal cancer (CRC) patients. Although the underlying mechanisms for differences caused by PTL remain still unclear, right-sided colon (RCC) and left-sided colon (LCC) are now considered as distinct entities because of their different molecular profile and clinical response to surgery and chemotherapy. In this article, we review the influence of PTL particularly on surgical management of primary and metastatic CRC settings. For nonmetastatic CRC, RCC could be a slightly superior prognostic factor after curative resection in stage I-II CRC, while RCC could be an inferior prognostic factor in stage III CRC with worse survival after recurrence, suggesting the oncological aggressiveness of recurrent RCC. For metastatic CRC, RCC could be a predictor of worse survival after hepatectomy of liver metastases from CRC with aggressive recurrence pattern and lower chance of re-resection. In lung metastases from CRC, the role of PTL still remains uncertain because of the limited number of studies. As to the impact of PTL on survival outcome after cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy for peritoneal metastases from CRC, a discrepancy exists among studies and further investigation will be needed. The very simple clinical factor of PTL could provide important information for the prediction of the survival outcome after surgery in CRC. Further clinical and basic research will facilitate the clinical application of PTL in a more specified and personalized manner.

***Keywords*:** Primary tumor location, colorectal cancer, surgery

Introduction

Despite recent progress in therapeutic and diagnostic modalities, colorectal cancer (CRC) is a serious public health problem worldwide owing to its high incidence and cancer-related mortality. It accounts for approximately 10% of diagnosed cancers with almost 900000 cancer-related deaths annually (*1*).

In recent years, the concept of primary tumor location (PTL) in CRC has attracted attention as a surrogate marker for predicting therapeutic effect and prognosis both in localized and metastatic disease settings. Right-sided colon cancer (RCC) and left-sided colon cancer (LCC) can be regarded as clinically and molecularly distinct entities. The differences between RCC and LCC could be explained to some extent by the fact that the right-sided colon and left-sided colon are embryologically different; the cecum to proximal two-thirds of the transverse colon arises from the midgut which is supplied by the superior mesenteric artery, while the distal third of the transverse colon to the upper two-thirds of the anorectal canal arises from the hindgut which is supplied by the inferior mesenteric artery (*2*).

Many previous studies reported the clinical and biological differences between RCC and LCC. For example, from the perspective of epidemiology, RCC patients are predominantly in females and at an older age, while LCC patients are predominantly in males and at an early age with a frequency of occurrence more than that of RCC (*3*). RCC patients also tend to have advanced and larger tumors (*3,4*), which may be explained by the asymptomatic features of RCC and the resulting delay in diagnosis. Pathologically, the proportion of poorly differentiated adenocarcinoma and mucinous adenocarcinoma, which are regarded as biologically aggressive histological types, is higher in RCC than in LCC (*3*). Also, the genomic aspects of RCC and LCC are substantially different. RCC is more often microsatellite instability-high (MSI-high) and CIMP-high phenotype, while LCC is more often chromosomal instability-high (CIN-high) phenotype (*5*). The mutation profiles of key oncogenes and tumor suppressor genes are also different between RCC and LCC. KRAS mutation, which is associated with the effectiveness of anti-EGFR therapy, is more frequent in RCC than in LCC (*6*). BRAF mutation, an inferior prognostic factor in stage IV CRC, is more often in RCC (*2*), while APC and TP53 mutations are more often in LCC (*2*). Recent basic research further

reported other various differences between RCC and LCC such as plasma protein expression profile (*7*). In addition to these clinicopathological and molecular differences, metastatic patterns between RCC and LCC are also different. It is reported that peritoneal metastases are most frequent in RCC patients, while lung metastases are most frequent in rectal cancer patients (*8*). For bone metastases, RCC patients have the lowest incidence and rectal cancer patients have the highest (*9*). These multifactorial differences caused by PTL might eventually lead to the prognostic differences between RCC and LCC.

Although systemic chemotherapy with targeted agents for CRC has made remarkable advances, surgical resection is still the gold standard for the treatment of CRC. Recently, many studies have reported the influence of PTL on surgical outcome of CRC in various clinical settings. In this article, we review the impact of PTL particularly on surgical management of primary and metastatic CRC.

Definition of PTL

The definition of PTL differs among studies and clinical trials. In practical settings, most studies define CRC proximal to splenic flexure as "right-sided" and CRC at or distal to splenic flexure as "left-sided". However, the most confusing point is that some studies exclude the rectum from the left-sided colon, while some studies include the rectum with the left-sided colon. Besides, transverse colon is sometimes excluded from the analysis due to its mixed embryologic origin. In this article, in order to avoid confusion, we classified CRC into two groups: RCC (from cecum to splenic flexure) and LCC (from splenic flexure to rectum), unless otherwise specified.

PTL and surgical outcome in nonmetastatic stage I - III CRC

Surgical resection is the first-choice treatment for nonmetastatic stage I-III CRC, with adjuvant chemotherapy for high-risk stage II and stage III CRC to improve survival outcome. Current clinical practice guidelines in Japan (*10*), US (*11*) and Europe (*12,13*) on the principals for surgical management of primary CRC are summarized in Table 1. Interestingly, the effects of PTL on prognostic outcomes after surgery between early (stage I and II) and advanced (stage III and IV) CRC patients are considered to be opposite. For

Table 1. Summary of current clinical practice guidelines on the principals for surgical management of primary and metastatic colorectal cancer

Guideline	Primary colorectal caner	Metastatic colorectal caner
Japanese Society for Cancer of the Colon and Rectum guidelines 2019 (*10*)	The extent of lymph node dissection is determined based on the preoperative clinical findings and on the extent of lymph node metastasis and depth of tumor invasion. The extent of the pericolic/perirectal lymph node in colon cancer is defined by the positional relationship between the primary tumor and the feeding artery. Metastasis of the pericolic/perirectal lymph node at a distance of 10 cm or more from the tumor edge is rare.	If both the distant metastases and the primary tumor are resectable, curative resection of the primary tumor performed, and resection of the distant me-tastases is considered. If the distant metastases are resectable but the primary tumor is unresectable, in principle, resection of the primary tumor and distant metastases is not performed, and another treatment method is selected. If the distant metastases are unresectable but the primary tumor is resectable, the indication for the resection of the primary tumor is determined, based on the clinical symptoms of the primary tumor and the impact on the prognosis.
NCCN guidelines Version 4.2020 (*11*)	The recommended surgical procedure for resectable colon cancer is an en bloc resection and adequate lymphadenectomy. Adequate pathologic assessment of the resected lymph nodes is important with a goal of evaluating at least 12 nodes. Patients with resectable T4b tumors or with bulky nodal disease may be treated with neoadjuvant systemic therapy prior to colectomy.	Patients with metastatic disease in the liver or lung should be considered for surgical resection if they are candidates for surgery and if all original sites of disease are amenable to resection (R0) and/or ablation. Six months of perioperative systemic therapy should be administered to patients with synchronous or metachronous resectable metastatic disease. When a response to chemotherapy would likely convert a patient from an unresectable to a resectable state (*i.e.*, conversion therapy), this therapy should be initiated.
ESMO consensus guidelines (*12,13*)	The resection should include a segment of colon of at least 5 cm on either side of the tumor. At least 12 lymph nodes should be resected when feasible. En bloc resection of adjacent organ-invaded portions must be car-ried out in case of pT4b.	In patients with clearly resectable disease and favorable prognostic criteria, perioperative treatment may not be necessary and upfront resection is justi-fied. In patients with technically resectable disease where the prognosis is unclear or probably unfavorable, perioperative combination chemotherapy should be administered. In potentially resectable patients (if conversion is the goal), a regimen leading to high response rates and/or a large tumor size reduction is recommended.

ESMO, European Society for Medical Oncology; NCCN, National Comprehensive Cancer Network.

example, a retrospective study from US in 2008 using The Surveillance, Epidemiology, and End Results (SEER) database including 77,978 CRC patients who underwent surgical resection of primary CRC showed that RCC patients had lower hazard ratio (HR) in overall survival (OS) in stage II CRC (HR = 0.91, 95% confidence interval [CI] 0.88-0.95, $P < 0.001$), while RCC patients had higher HR in OS in stage III (HR = 1.06, 95% CI 1.02-1.11) and stage IV CRC (HR = 1.22, 95% CI 1.16-1.28). No difference in OS between RCC and LCC was observed in stage I CRC (*4*). A study from US in 2019 including 114,839 stage I-II and 71,024 stage III CRC patients, who underwent surgical resection, reported similar results. In stage I-II CRC, RCC patients had superior OS (HR = 1.13, 95% CI 1.09-1.17, $P < 0.001$). In stage III CRC, LCC patients had superior OS with chemotherapy (HR = 0.85, 95% CI 0.815-0.892, $P < 0.001$), while no difference in OS without chemotherapy (HR = 0.97, 95% CI 0.912-1.02, $P = 0.18$) (*14*). The tendency that early stage RCC patients had a survival benefit compared with early stage LCC patients may be partially explained by the greater prevalence of MSI-high in RCC, which predicts a good survival outcome (*15*).

In 2018, Ishihara *et al.* from Japan retrospectively investigated 5,664 stage II and III CRC patients who underwent curative resection. Although the 5-year recurrence free survival was slightly superior in RCC than in LCC (83.9% versus 81.1%, $P = 0.019$), the 5-year cancer-specific survival (CSS) after recurrence was significantly inferior in RCC than in LCC (30.6% versus 43.6%, $P = 0.016$), suggesting the oncologically aggressive nature of RCC after recurrence (*16*). The same tendency that the effect of PTL in stage II and III CRC might be related to survival outcome after recurrence rather than the recurrence risk after surgery with or without adjuvant chemotherapy was confirmed in several other studies. In 2016, Kerr *et al.* from UK analyzed the data from two randomized trials regarding adjuvant chemotherapy with 1,935 stage II and III CRC patients who received surgery, and reported that PTL had no significant effect on relapse-free survival, while RCC patients had significantly inferior survival after recurrence compared with LCC patients (HR 1.53, 95% CI 1.14-2.06, $P = 0.004$) (*17*). In 2019, Cascinu *et al.* from Italy analyzed data from three randomized trials including 5,239 stage II and III CRC patients who underwent surgical resection. In this study, the authors classified CRC into three groups: RCC (from the cecum to the hepatic flexure), transverse colon, and LCC. They found that there was no difference both in disease-free survival (DFS) and OS in stage II patients, while RCC patients had similar DFS but significantly inferior OS compared to LCC patients in stage III patients (HR = 1.35, 95% CI 1.14-1.62, $P < 0.001$) (*18*). A retrospective study from Japan in 2020 investigated 9,194 stage III CRC patients who received surgical resection. In this study, the authors classified the CRC patients into three groups: RCC, LCC (from the splenic flexure to sigmoid colon), and rectal cancer. They reported that rectal cancer was associated with worse relapse-free survival compared to LCC and RCC, while RCC had significantly shorter 5-year OS after recurrence compared to LCC and rectal cancer (RCC: 23.3%, LCC: 36.6%, rectal cancer: 31.6%, $P < 0.001$). They also reported the difference in recurrence pattern among PTL; 20% of RCC were peritoneal dissemination, 42% of LCC were liver metastases, and 33% of rectal cancer were local recurrence, which may influence survival outcomes after recurrence (*19*).

The results of large cohort studies on PTL and surgical outcomes after resection for stage I-III CRC are summarized in Table 2.

PTL and metastatic stage IV CRC

For resectable stage IV CRC patients, surgical resection of primary and metastatic lesions is actively considered aiming at long-term disease control and the possibility of cure. Current clinical practice guidelines in Japan (*10*), US (*11*) and Europe (*12,13*) on the principals for surgical management of metastatic CRC are summarized in Table 1. On the other hand, for the unresectable advanced stage IV CRC patients, systemic chemotherapy is the standard treatment while considering the possibility of resection of primary and metastatic disease in the case of sufficient tumor shrinkage. At the present time, with the combination of chemotherapy and targeted agents, the median OS in unresectable CRC patients has improved over time and now ranges from 25 to 30 months (*20*). In unresectable metastatic CRC, several studies have demonstrated that PTL could be both the prognostic marker for survival and the predictive marker for the therapeutic response to molecular targeted agents. The CALGB/SWOG 80,405 trial was a randomized clinical trial, which compared the effect of bevacizumab with cetuximab added to first-line FOLFOX or FOLFILI in metastatic CRC patients (*21*). The post-hoc study of this trial showed that the median OS of LCC patients was significantly superior to that of RCC (cecum to hepatic flexure, transverse colon was excluded in this study) patients (33.3 versus 19.4 months, $P < 0.0001$). Moreover, among KRAS wild-type metastatic CRC patients, RCC patients had longer OS from bevacizumab than cetuximab (HR 1.36, 95% CI 0.93-1.99, $P = 0.10$), whereas LCC patients had longer OS from cetuximab than bevacizumab (HR 0.77, CI 0.59-0.99, $P = 0.04$) (*21*). Similar results were reported in other randomized clinical trials such as CRYSTAL and FIRE-3 demonstrating that RAS wild-type metastatic RCC patients had limited benefit from first-line FOLFIRI plus cetuximab (*22*). The PRIME study also reported that the addition of panitumumab to FOLFOX improved the OS of LCC patients but not RCC patients (*23*). The current consensus is that RAS wild-type metastatic RCC patients

Table 2. Summary of large cohort studies on PTL and surgical outcomes after resection for Stage I - III CRC

Year	Reference	Study type	Study period	Number of patients	Survival analysis for RCC versus LCC
2008	Meguid *et al.* (*4*)	Retrospective (SEER database)	1988-2003	77,978	Stage I: no difference in OS Stage II: superior in OS (HR 0.91, 95% CI 0.88-0.95) Stage III: inferior in OS (HR 1.06, 95% CI 1.02-1.11)
2016	Kerr *et al.* (*17*)	Retrospective (Two trials)	2002-2004 2005-2010	1,935	Stage II-III: no difference in RFS, but inferior in OS after recurrence (HR 1.53, 95% CI 1.14-2.06)
2018	Ishihara *et al.* (*16*)	Retrospective (22 centers)	1997-2006	5,664	Stage II-III: superior in RFS, but inferior in 5-year CSS after recurrence (30.6% versus 43.6%)
2019	Turner *et al.* (*14*)	Retrospective (NCDB)	2006-2013	185,863	Stage I-II: superior in OS (HR 0.88, 95% CI 0.85-0.92) Stage III with chemotherapy: inferior in OS (HR 1.18, 95% CI 1.12-1.22) Stage III without chemotherapy: no difference in OS
2019	Cascinu *et al.* (*18*)	Retrospective (Three trials)	1989-1992 1992-1998 2007-2013	5,239	Stage II: no difference in DFS and OS Stage III: no difference in DFS, but inferior in OS (HR 1.35, 95% CI 1.14-1.62)
2020	Shida *et al.* (*19*)	Retrospective (24 centers)	1997-2012	9,194	Stage III: inferior in 5-year OS after recurrence (RCC: 23.3%, LCC: 36.6%, rectal cancer: 31.6%)

CI, confidence interval; CRC, colorectal cancer; CSS, cancer specific survival; DFS, disease free survival; HR, hazard ratio; LCC, left-sided colon cancer; NCDB, The National Cancer Database; OS, overall survival; PTL, primary tumor location; RCC, right-sided colon cancer; RFS, recurrence free survival; SEER, surveillance, epidemiology, and end results.

could have limited benefit from the anti-EGFR therapy. At present, in the NCCN guidelines version 4.2020, the anti-EGFR agents (cetuximab or panitumumab) in first-line therapy for advanced or metastatic CRC are recommended only for KRAS/NRAS/BRAF wild-type LCC (*11*).

PTL and surgical outcome in liver metastases from CRC

The liver is the most frequent site of distant metastases in CRC. Approximately 15% to 20% of CRC patients present with liver metastases at first diagnosis and more than 50% of CRC patients will develop liver metastasis during the course of the disease, accounting for two-thirds of CRC deaths (*24*). For resectable liver metastasis from CRC, surgical resection is the gold standard for prolonging progression-free survival (PFS) and potentially cure. At present, the reported 5-year OS after resection of liver metastasis from CRC approaches 40% to 50 % with the advent of effective chemotherapy regimen and advances in surgical techniques (*25-27*). In addition, the initially unresectable liver metastasis at the time of presentation could be down-staged and resected by conversion surgery with modern systemic chemotherapy including molecular target agents. For example, in a recent systemic review, the combination of fluorouracil, oxaliplatin, and irinotecan plus bevacizumab (FOLFOXIRI-Bev) achieved an overall surgical conversion rate of 39.1% and a R0 resection rate of 28.1% with median PFS and OS of 12.4 months and 30.2 months, respectively (*28*).

In recent years, many papers reported the effect of PTL on the prognosis after hepatectomy for liver metastases from CRC. A retrospective SEER database study from China in 2019 that investigated 1,508 CRC patients with synchronous liver metastases who underwent R0 surgical resection showed that RCC patients had significantly worse OS and CSS compared to LCC patients (OS, HR = 1.75, 95% CI 1.34-2.29; CSS, HR = 1.76, 95% CI 1.33-2.35) (*29*). Another retrospective SEER database study from US in 2020 demonstrated that both LCC (from the splenic flexure to rectosigmoid junction in this study) and rectal cancer patients with synchronous liver metastasis who underwent R0 surgical resection had significantly improved OS (HR = 0.72, 95% CI 0.62-0.83, $P < 0.001$) and disease-specific survival (HR = 0.73, 95% CI 0.58-0.92, $P = 0.008$) compared to RCC patients, while there was no survival difference between LCC and rectal cancer patients (*30*). This tendency that RCC is a poor prognostic factor has also been demonstrated in several systematic reviews. A meta-analysis from China in 2019 reviewing 12 studies with 6,387 CRC patients with liver metastases who underwent hepatic resection showed that RCC patients had worse 5-year OS (HR = 1.354, 95% CI 1.238-1.482) compared to LCC patients, but no significant difference in 5-year DFS (HR = 1.104, 95% CI 0.987-1.235) (*31*). Similarly, another meta-analysis from China in 2019 reviewing 45 study cohorts with 21,953 patients reported that RCC patients had significantly worse OS (HR = 1.39, 95% CI 1.28-1.51, $P < 0.001$) and DFS (HR = 1.18, 95% CI 1.06-1.32, $P = 0.004$) compared to LCC patients (*32*). For patients who need extensive liver resection, portal vein embolization (PVE) is widely used before surgery to promote the

growth of the future liver remnant (*33*). Although the number of patients was limited (N = 59), a retrospective study from Germany in 2020 reported that PTL was the only statistically significant predictor of intrahepatic PFS after PVE subsequent to major hepatic surgery on Cox regression analysis (HR = 2.242, 95% CI 1.125-4.465, P = 0.022), and RCC patients had significantly shorter intrahepatic PFS compared to LCC patients (median 4.0 months versus 10.2 months, P = 0.018) (*34*). Furthermore, the patterns of recurrence between RCC and LCC after resection of liver metastases may also be different. In 2020, Russolillo *et al.* from Italy reported that recurrence after hepatectomy in RCC patients was more often encephalic and at multiple sites, and RCC patients had a lower chance of re-resection compared to LCC patients (27.9% versus 37.5%, P = 0.024) (*35*). Overall, RCC could be an independent predictor of worse survival after surgical resection of liver metastases from CRC. Thus, the stratification based on PTL would be useful in clinical practice such as adding adjuvant chemotherapy and careful surveillance after hepatectomy of liver metastases from RCC.

PTL and surgical outcome in lung metastases from CRC

The lung is the second most frequent target organ of metastasis from CRC. A total of 5% to 15% of CRC patients will develop lung metastases. For unresectable lung metastases from CRC, systemic chemotherapy is considered, though the conversion rate is very low compared to liver metastases (*36*). Despite the lack of randomized controlled studies, in selected patients with resectable disease, pulmonary metastasectomy is widely considered for the treatment of lung metastases from CRC (*37*). According to a review that investigated 21 studies with 8,361 CRC patients with lung metastases who underwent surgical resection, the 5-years OS after first pulmonary metastasectomy were 24 to 82% and the median OS ranged from 35 to 70 months (*38*). Another meta-analysis investigated 15 studies with 1,669 CRC patients with lung metastases reported a mean 5-year OS of 49% (range 25-72%) after pulmonary metastasectomy (*39*). Various prognostic factors affecting survival outcomes after pulmonary metastasectomy have been reported. A best evidence topic review in 2016 reported that the prognostic factors in pulmonary metastasectomy for lung metastases from CRC include the size and number of metastases, intra-thoracic lymph node involvement, pre-thoracotomy CEA levels, and response to induction chemotherapy (*40*).

Regarding PTL, few studies investigated the effect of PTL on survival outcomes after pulmonary metastasectomy for lung metastases from CRC. One retrospective study from the US in 2020 with 194 CRC patients with lung metastases reported that LCC (from splenic flexure to sigmoid colon in this study) patients experienced prolonged 5-year OS after surgical resection on multivariate analysis compared to rectal cancer (HR = 0.31, 95% CI 0.10-0.93, P = 0.036), while no significant difference was observed between LCC and RCC (*41*). Further study with a large patient cohort will be needed to elucidate the impact of PTL on the outcome of surgical resection for lung metastases from CRC.

PTL and surgical outcome in peritoneal metastases from CRC

The peritoneum is the third most frequent site for metastases from CRC. About 4% to 5% of all CRC patients present with synchronous peritoneal metastases (*42,43*). Historically, peritoneal metastases from CRC had the worst outcome. However, with the advent of an aggressive surgical treatment in the form of complete cytoreductive surgery (CRS) plus hyperthermic intraperitoneal chemotherapy (HIPEC), the prognosis of CRC patients with peritoneal metastasis has been improved. At present, the expected median OS and 5-year survival obtained by CRS plus HIPEC at high volume centers are 19.2-34 months and 19-48%, respectively (*43*). Thus, in the selected patients with resectable peritoneal metastases from CRC, surgical resection particularly with CRS plus HIPEC is now actively attempted with potential for long-term survival and cure. On the other hand, the efficacy of HIPEC for peritoneal metastases from CTC still remains controversial according to the recent PRODIGE7 trial. The PRODIGE7 trial was the first randomized phase III trial that evaluated the effectiveness of HIPEC with oxaliplatin for the treatment of CRC patients with peritoneal metastases (*44*). This trial randomized 265 CRC patients with peritoneal metastases (peritoneal cancer index score ≤ 25) to CRS alone or CRS plus HIPEC with oxaliplatin. It reported excellent median OS of 41.7 months in non-HIPEC group and 41.7 months in HIPEC group, but there was no difference in OS between the groups (HR = 1.00, 95% CI 0.73-1.37, P = 0.995). The role of HIPEC for the treatment of peritoneal metastases from CRC should be studied further.

The well-known prognostic factors affecting survival after CRS plus HIPEC are peritoneal cancer index score and completeness of cytoreduction score. In addition to these factors, several studies investigated the prognostic role of PTL for peritoneal metastases from CRC. A retrospective population-based cohort study from the Netherlands in 2020 included 7930 CRC patients with synchronous peritoneal metastases. Of all 7,930 patients, 564 patients (7.1%) received CRS plus HIPEC. The overall analysis including all 7,930 CRC patients with peritoneal metastases showed that RCC was an independent prognostic factor in multivariate analysis and was significantly associated with worse OS compared to LCC (HR 1.11, 95% CI 1.03-1.19, P =

Table 3. Summary of studies on PTL and surgical outcomes after CRS plus HIPEC for peritoneal metastases from CRC

Year	Reference	Study type	Study period	Number of patients	Survival analysis for RCC versus LCC
2019	Kelly *et al.* (*48*)	Retrospective (Three centers)	1992-2016	115	Inferior in DFS (HR 2.27, 95% CI 1.09-4.76), inferior in OS (HR 2.57, 95% CI 1.13-5.84)
2019	Kotha *et al.* (*47*)	Retrospective (12 centers)	2000-2017	336	Inferior in DFS (HR 1.75, 95% CI 1.19-2.56), inferior in OS (HR 1.72, 95% CI 1.09-2.73)
2019	Péron *et al.* (*46*)	Retrospective (16 centers)	2004-2017	796	No difference in PFS (HR 1.02, 95% CI 0.85-1.23) and OS (HR 0.99, 95% CI 0.79-1.23).
2020	Blakely *et al.* (*49*)	Retrospective (CCR)	2004-2012	272	Inferior in DFS (median 21 versus 41 months, $P = 0.0011$) Inferior in OS (median 15.5 versus 34 months, $P = 0.0010$)
2020	de Boer *et al.* (*45*)	Retrospective (Population-based)	1995-2016	564	No difference in OS (median 30.3 versus 34.6 months, $P = 0.301$)

CCR, California Cancer Registry; CI, confidence interval; CRC, colorectal cancer; CRS, complete cytoreductive surgery; DFS, disease free survival; HIPEC, hyperthermic intraperitoneal chemotherapy; HR, hazard ratio; LCC, left-sided colon cancer; OS, overall survival; PFS, progression free survival; PTL, primary tumor location; RCC, right-sided colon cancer.

0.007). However, in the subgroup analysis limited to 564 patients who underwent CRS plus HIPEC, OS of RCC patients did not significantly differ from that of LCC patients (*45*). Another retrospective study from France in 2019 with 796 patients with peritoneal metastases from CRC who were treated with CRS plus HIPEC also reported the same tendency that there was no significant difference in OS or PFS between RCC and LCC (median OS 3.5 versus 4.0 years, HR = 0.99, 95% CI 3.5-4.4 years, $P = 0.90$) patients (*46*).

On the other hand, other retrospective studies with a smaller patient population reported worse prognosis of RCC after CRS plus HIPEC. A retrospective cohort study from the US in 2019 with 336 patients with peritoneal metastases from CRC reported a significantly shorter OS for RCC compared to LCC (median 30 months versus 45.4 months, $P = 0.028$), and RCC was an independent predictor of worse DFS and OS on multivariate analysis (*47*). A retrospective study from the US in 2019 with 115 patients with peritoneal metastases from CRC who underwent CRS plus HIPEC showed the same result of significantly inferior DFS (HR = 2.27, 95% CI 1.09-4.76, $P = 0.029$) and OS (HR = 2.57, 95% CI 1.13-5.84, $P = 0.021$) in RCC compared to LCC (*48*). Another retrospective study from US in 2020 with 272 patients with peritoneal metastases from CRC who underwent CRS plus HIPEC showed the same result of significantly shorter DFS and OS in RCC compared to LCC (15.5 months versus 34 months, $P = 0.0010$) (*49*).

The results of studies on PTL and surgical outcomes after CRS plus HIPEC for peritoneal metastases from CRC are summarized in Table 3. Given the differing results between these studies, it seems that the PTL may affect the prognosis of peritoneal metastases from CRC, and RCC patients have worse survival than LCC patients, while the impact of PTL on surgical outcome particularly with CRS plus HIPEC still remains controversial.

PTL and other distant metastases from CRC

Although not related to surgical management, a few studies reported the effect of PTL on other distant metastases such as brain or bone metastases. A recent retrospective SEER database study from China in 2020 investigated a total of 202,401 CRC patients. In this study, CRC was classified into three groups: RCC, LCC (from the splenic flexure and rectosigmoid junction), and rectal cancer. The reported overall incidence of brain or bone metastasis at initial diagnosis was 1.38% and 6.12% in metastatic CRC patients, respectively. PTL was associated with the incidence of bone metastasis with the lowest incidence for RCC (4.69%) and the highest incidence for rectal cancer (8.56%), while not associated with that of brain metastasis. As to prognosis, as with liver metastases, RCC patients had the shortest median survival in both brain (3 months) and bone metastasis (4 months) compared to LCC and rectal cancer patients (*9*).

Conclusion

Although the reason for the differences caused by PTL remains still unclear and probably multifactorial, the current understanding is that RCC is significantly associated with inferior survival after surgical resection compared to LCC in locally advanced CRC and liver metastases from CRC. In lung metastases from CRC, the role of PTL still remains uncertain because of the limited number of studies. Regarding peritoneal metastases from CRC, the role of PTL still seems controversial and needs further study to clarify the effect of PTL on surgical management. The very simple clinical factor of PTL in CRC could be an important biomarker for predicting the therapeutic outcome of surgical resection of primary and metastatic CRC. Further clinical and basic research will facilitate the clinical application of PTL in a more specified and personalized manner.

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Validation of mailed *via* postal service dried blood spot cards on commercially available HIV testing systems

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Abstract: The demand for HIV testing using dried blood spots (DBS) has increased recently. However, DBS is not an approved sample for HIV testing in Japan. This study examined the validation of HIV testing with DBS, prepared at the laboratory or remotely and mailed *via* postal service to the laboratory. DBS were punched out from a 5.5 mm diameter circle on filter paper, then eluted with 600 μL of phosphate buffered saline overnight at 4°C, and analyzed by Lumipulse S HIVAg/Ab (LUM). The mean LUM count of DBS was 237.4-times diluted compared to titrated plasma. Repeated sample testing showed that although LUM count of DBS decreased slightly with increase in sample storage time (up to one month), it did not affect the result of HIV testing with DBS. Based on testing of 50 HIV+ confirmed cases and 50 HIV- persons, the estimated sensitivity was 98% (49/50) with a specificity of 100% when the cut-off value is 0.5. The single false negative case was a patient with undetectable viral load over the last 10 years, resulting in a decrease of antibody titer below the cut-off level. In conclusion, although DBS cannot completely replace plasma in HIV testing because the sensitivity was a little lower than that of plasma, it can be potentially useful for a screening test by self-finger-prick and postal service use. This will allow people to receive HIV testing without visiting public health centers.

***Keywords*:** HIV, dried blood spot, self-finger-prick, postal service

Introduction

In Japan and many other countries, HIV testing is conducted free of charge at local public health centers. However, some centers are not always convenient, for example, operating over limited hours during the week or closed over the weekends. For these reasons, the number of HIV tests conducted at centers has been on the decline by about 30% in the last decade according to the Ministry of Health, Labour and Welfare, Japan (*1*). Furthermore, during the second quarter of 2020 when the first wave of the COVID-19 pandemic hit Japan, HIV testing decreased steeply by 70% compared with the same quarter of the previous year (*2*) due to both the increased load on medical staff at public health facilities due to COVID-19 and lockdowns imposed on people. Under these circumstances, it is important to design alternative HIV testing procedures that can be performed without visiting public health centers (*3*).

In this regard, HIV testing using mailed *via* postal service samples has increased annually during the last several years, with an estimated rise in 2019 of 14.5% (according to Sudo K. 34th meeting of the Japanese Society for AIDS Research, 2020; P-S5-2). Many of them seem to use dried blood spot (DBS) samples because they are easy to collect by oneself, transport, and store (at ambient temperature), compared with fresh blood samples (*4*). However, DBS is currently not approved as a sample for HIV testing in Japan; only whole blood or serum or plasma samples are approved. Thus, validation and approval of HIV testing with DBS is urgently needed in Japan.

Many studies have described the use of DBS in HIV testing, however, these were often collected by medical staff who could obtain a sufficient volume of blood (*5-10*). In comparison, self-finger-prick sometimes yields a small volume of blood that is too inadequate to fill the circle on the designated area on the Whatman 903 card (*11*), which has been used in most HIV/DBS studies. Though these studies explored concordance rate of HIV testing between plasma and DBS, they did not focus on fundamental validation such as difference of filter papers, carry-over, accuracy, stability, or dilution effect in the elution step.

The purpose of this study was to validate HIV testing with DBS samples derived from self-finger-prick/postal service on the above fundamental points.

Materials and Methods

Participants and sample collection

For the fundamental research part of the study, 3 mL of venous blood was obtained from 50 HIV+ patients at AIDS Clinical Center and from 30 men who had sex with men (MSM) followed at the sexual health clinic and confirmed to be HIV-negative at the National Center for Global Health and Medicine (NCGM). Medical staff prepared DBS samples from these individuals by pipetting drops of collected venous blood onto filter paper. We also asked 20 healthy volunteers to prepare DBS by self-finger-prick using the BD Microtainer® contact-activated lancet Medium flow (Becton, Dickinson and Company, Franklin Lakes, NJ). After overnight drying, all DBS samples, including those prepared from the HIV+ and HIV- individuals, were mailed *via* postal service to our laboratory. All DBS samples were anonymously labelled and thus the laboratory staff was blinded to the clinical status of the individuals who provided the samples.

The study was approved by the Human Ethics Committee of the NCGM (#NCGM-G-2065), and each provider of blood samples also signed informed consent in accordance with the Declaration of Helsinki. The 20 healthy volunteers were considered to have consented to the study by mailing *via* postal service the DBS samples. These samples were collected from December 2017 to May 2018.

Preparation of DBS

For comparison of the filter papers used for DBS, we tested the following 9; Filter paper for blood collection (EIKEN CHEMICAL, Tokyo, Japan), DENTAL STICK II (SANRITSU, Chiba, Japan), 903 Protein Saver Card (GE Healthcare Life Sciences, Marlborough, MA), 903 Protein Saver Snap Apart Card (GE Healthcare Life Sciences), FTA™ DMPK-A Card (GE Healthcare Life Sciences), FTA™ DMPK-B Card (GE Healthcare Life Sciences), FTA™ DMPK-C Card (GE Healthcare Life Sciences), FTA™ Classic Card (GE Healthcare Life Sciences), and NucleoCard (MACHEREY-NAGEL, North Rhine-Westphalia, Germany).

HIV testing using DBS

A tool was used to punch out 5.5 mm diameter circles from the DBS filter paper (CARL, Tokyo, Japan). After punching out the DBS, the operator also punched out blank parts of the filter paper at three spots to avoid any contamination before using the punching tool again. The punched-out DBS were eluted overnight with 600 μL of phosphate-buffered saline (PBS) pH 7.2 (ThermoFisher Scientific, Waltham, MA) at 4°C. After vortexing and centrifugation, 100 μL of the supernatants were analyzed by Lumipulse S HIVAg/Ab (FUJIREBIO, Tokyo, Japan) (LUM). The LUM count represented the linear regression between 0.1 and 15.0, with a cutoff value for HIV positivity of ≥ 1.0 using the standard method. Samples determined as HIV-positive were tested again by the HISCL™5000 HIV Ag+Ab (Sysmex, Hyogo, Japan) (HIS) using 30 μL of the remaining supernatant. The HIS score represented the linear regression between 0.0 and 100, with a cutoff value for HIV positivity of ≥ 1.0 using the standard method. Both LUM and HIS were chemiluminescent enzyme immunoassay systems of the 4th generation HIV antigen and antibody testing approved in Japan for serum or plasma samples. In contrast, not only in Japan but also in other countries, DBS are not approved as a sample for LUM and HIS.

Statistical analysis

Bivariate correlation analysis was conducted by IBM SPSS Statistics version 23 (IBM, Armonk, NY) with a significance of $p < 0.05$. Simple linear regression was plotted by GraphPad Prism version 8.3.0 (GraphPad Software, San Diego, CA).

Results

Comparison of filter papers

First, we compared the performance of the nine filter papers using LUM. We prepared DBS using whole blood samples obtained from seven HIV+ patients (samples A-G, Figure 1). LUM count was similar for all filter papers, except the FTA™ DMPK-B card. We eventually adopted the Filter paper for blood collection (EIKEN CHEMICAL) throughout this study because of its design of four spots on the filter paper, each requiring 25 μL blood, which matches the volume of blood obtained from a self-finger-prick.

Carry-over

To assess the probability of carry-over, the DBS and the three blank circles of the filter paper were punched out and subjected to LUM. In this test, we used 6 HIV+ samples, and no carry-over was observed even in the first punched out blank filter paper (Table 1). Subsequent sampling for analyses used all 3 blank punches after each DBS.

Comparison of blood volume

To determine the appropriate amount of whole blood necessary, 1, 2, 4, 8, 10, and 20 μL of whole blood were pipetted on the filter paper discs prior to the LUM measurement procedure. In this part of the study, we used samples from 7 HIV+ individuals. LUM count

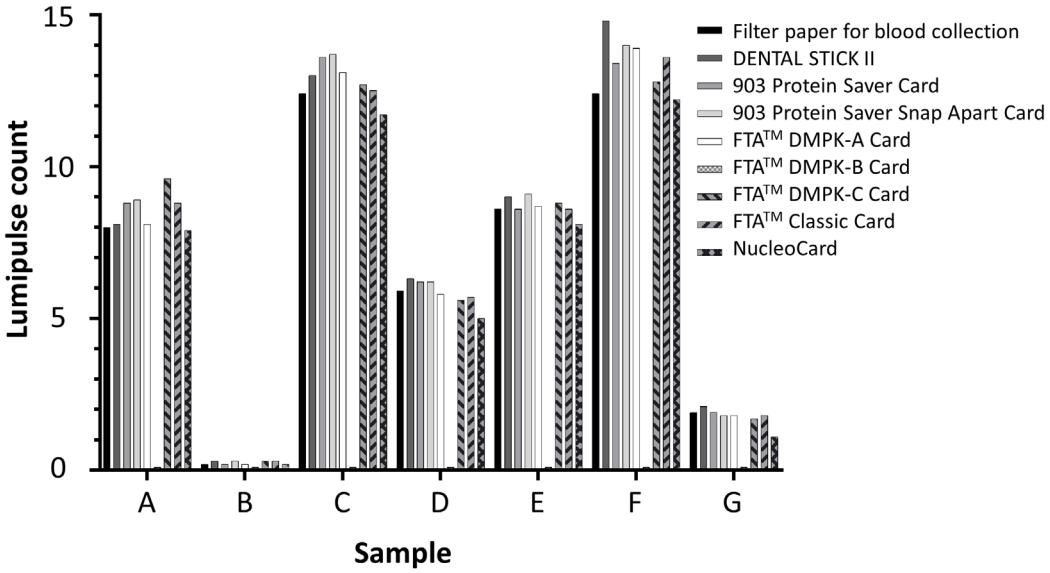

Figure 1. Comparison of Lumipulse S HIVAg/Ab among 9 filter papers. Blood samples were obtained from 7 HIV+ donors, then whole blood was placed on nine filter papers using a pipette. Each DBS was punched, eluted with PBS, and analyzed by Lumipulse S HIVAg/Ab.

Table 1. Carry-over in continuous punch of DBS with Lumipulse S HIVAg/Ab

Sample	Lumipulse count			
	DBS	Blank 1	Blank 2	Blank 3
H	15.0	0.1	0.1	0.1
I	15.0	0.1	0.1	0.1
J	2.2	0.1	0.1	0.1
K	4.0	0.1	0.1	0.1
L	15.0	0.1	0.1	0.1
M	15.0	0.1	0.1	0.1

DBS: dried blood spot.

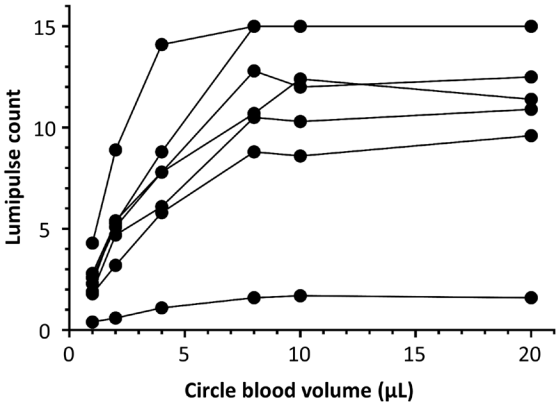

Figure 2. Comparison of Lumipulse S HIVAg/Ab among different volumes of blood placed on the filter paper using a pipette. The blood samples were obtained from 7 HIV+ donors, then 1, 2, 4, 8, 10, and 20 μL of whole blood were placed on the filter papers using a pipette. Each DBS was punched, eluted with PBS, and analyzed by Lumipulse S HIVAg/Ab.

increased with increasing amounts of blood up to 8 μL but reached a plateau thereafter (Figure 2). These results indicated that 8 μL of whole blood was sufficient to spread throughout the entire 5.5 mm circle on the filter card. Since less than 8 μL of blood may be collected in real sampling situations, we decided that testing criteria should exclude samples that appear to contain < 8 μL of blood (as judged by DBS diameter).

Comparison between DBS and plasma

To determine the difference in sensitivity between DBS and plasma, we compared the LUM count of DBS with that obtained from plasma samples after serial titrations. First, whole blood was used to create DBS, then plasma was prepared from the same blood sample. The samples were serially diluted 100, 300, 1000, 3000, and 10000 times with PBS. We used 5 HIV+ samples in this experiment. LUM counts of DBS corresponded to 265.5, 216.8, 238.8, 257.8, and 208.3 times dilution (mean of 237.4) of the titrated plasma samples (Table 2).

Accuracy and stability

To evaluate the accuracy of our method, we tested the within-run accuracy. First, we prepared 10 DBS samples from a single whole blood sample, then analyzed them with LUM. Five HIV+ samples were used in this experiment. The mean coefficient of variance was 6.69% (range 4.22-10.14 %) (Table 3). Second, we tested the between-run accuracy. We prepared 5 DBS samples from a single whole blood sample, stored them at room temperature and analyzed them by LUM on days 0, 6-16, 13-18, 21-30 and 28-37. The mean coefficient of variance was 8.74% (range, 4.38-12.28%), which was similar to that of the within-run accuracy experiment

Table 2. Count of Lumipulse S HIVAg/Ab with diluted plasma and DBS

Sample	Lumipulse count of diluted plasma					Lumipulse count of DBS	DBS/plasma ratio
	1/100	1/300	1/1000	1/3000	1/10000		
N	(15.0)*	7.8	2.8	0.8	0.2	8.9	1/265.5
O	3	1.3	0.5	0.2	0.1	1.5	1/216.8
P	(15.0)*	9.0	3.3	0.9	0.3	11.4	1/238.8
Q	(15.0)*	8.9	2.9	0.8	0.3	10.4	1/257.8
R	(15.0)*	9.6	3.3	0.9	0.3	13.9	1/208.3

The DBS/plasma ratio was calculated by linear regression of Lumipulse count with diluted plasma. *We excluded these from linear regression analysis because 15.0 was the upper limit of Lumipulse count. DBS: dried blood spot.

Table 3. Within-run accuracy of Lumipulse S HIVAg/Ab with DBS

Sample	*n*	Mean Lumipulse count	Range	Standard deviation	Coefficient of variance (%)
S	10	4.91	4.5 - 5.3	0.251	5.11
T	10	8.76	8.3 - 9.4	0.369	4.22
U	10	0.90	0.8 - 1.0	0.045	4.97
V	10	1.54	1.3 - 1.9	0.156	10.14
W	10	6.84	6.0 - 8.0	0.617	9.02

DBS: dried blood spot.

Table 4. Between-run accuracy of Lumipulse S HIVAg/Ab with DBS

Sample	*n*	Mean Lumipulse count	Range	Standard deviation	Coefficient of variance (%)	Slope (Lumipulse count/day)	*p* value of slope
X	5	13.46	12.6 - 15.0	0.967	7.18	-0.0685	0.0379
Y	5	9.76	9.2 - 10.4	0.427	4.38	-0.0145	0.5153
Z	5	0.82	0.7 - 0.9	0.075	9.13	-0.0012	0.8016
AA	5	1.08	0.9 - 1.3	0.133	12.28	-0.0093	0.0472
AB	5	5.54	4.8 - 6.3	0.595	10.74	-0.0387	0.1014

The slopes and *p* values of the slope were calculated by linear regression analysis. DBS: dried blood spot.

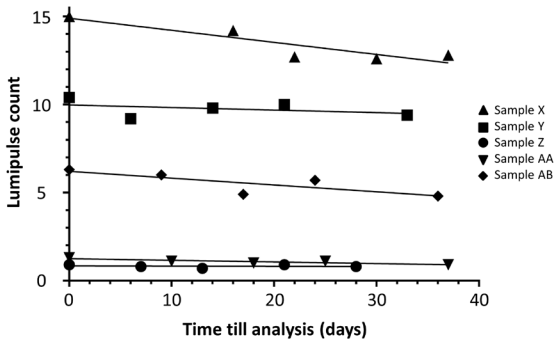

Figure 3. Stability test of Lumipulse S HIV Ag/Ab with DBS samples. Blood samples were obtained from 5 HIV+ donors. Five DBS were prepared from each using whole blood. They were stored at room temperature and analyzed by Lumipulse S HIV Ag/Ab after 0, 6-16, 13-18, 21-30 and 28-37 days.

(Table 4). Although a small but gradual decrease in LUM count was noted in two samples (Figure 3), LUM could be counted in all samples up to day 28.

Sensitivity and specificity

To determine the sensitivity and specificity of our method, we tested 50 samples from HIV+ and HIV- persons. HIV was normally considered positive using a LUM cutoff value of 1.0 for plasma samples. In this study, however, we lowered the cutoff value to 0.5 because we found that DBS samples were diluted around 150 times with PBS due to DBS processing. Next, we re-tested all samples with LUM value ≥ 0.5 by HIS using a lower cutoff value of 0.5 (standard cutoff value = 1.0). Among 50 HIV+ DBS samples, 49 samples were judged as HIV positive (sensitivity: 98%). The LUM counts ranged from 0.2 to 15.0 (Figure 4A). HIS counts of the LUM-positive samples ranged from 0.1 to 111.4 (Figure 4B), resulting in 3 samples being considered negative for HIV by HIS. These three samples with HIS counts of 0.1, 0.2, and 0.3 corresponded to LUM counts of 0.2, 2.6, and 0.8, respectively. None of 50 HIV- DBS samples were judged as HIV positive by LUM (specificity: 100%). LUM count was 0.1 in all HIV-negative DBS samples. One HIV+ patient whose LUM count was 0.2 has been on anti-retroviral treatment (ART) for more than 10 years with persistently undetectable viral load during the same period. We tested a plasma sample from this false-negative patient by western blotting (NEW LAV BLOT 1, Bio-Rad Laboratories, Tokyo). The results showed

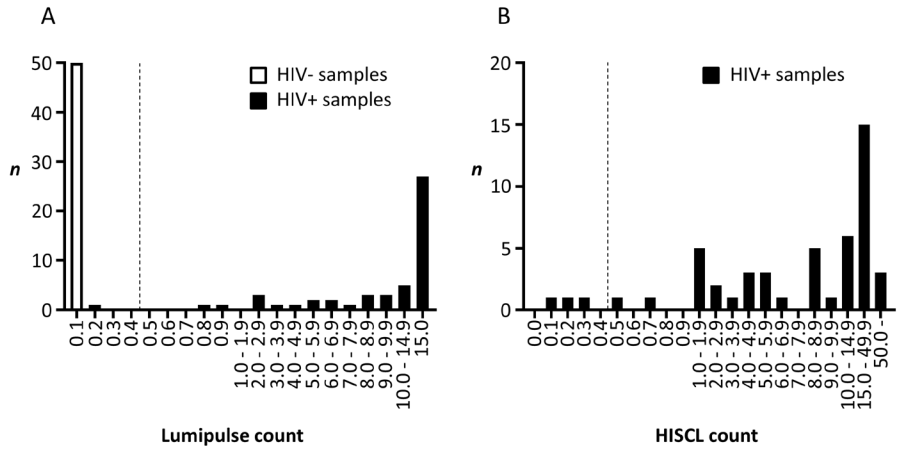

Figure 4. Specificity and sensitivity test of Lumipulse S HIVAg/Ab and HISCL™-5000 HIV Ag+Ab using DBS samples. The DBS samples were obtained from 50 HIV+ and 50 HIV- donors. **(A)** 100 DBS samples were analyzed by Lumipulse S HIVAg/Ab. Dotted line: cutoff value. **(B)** 50 DBS samples from HIV+ donors were analyzed by HISCL™-5000 HIV Ag+Ab. Dotted line: cutoff value.

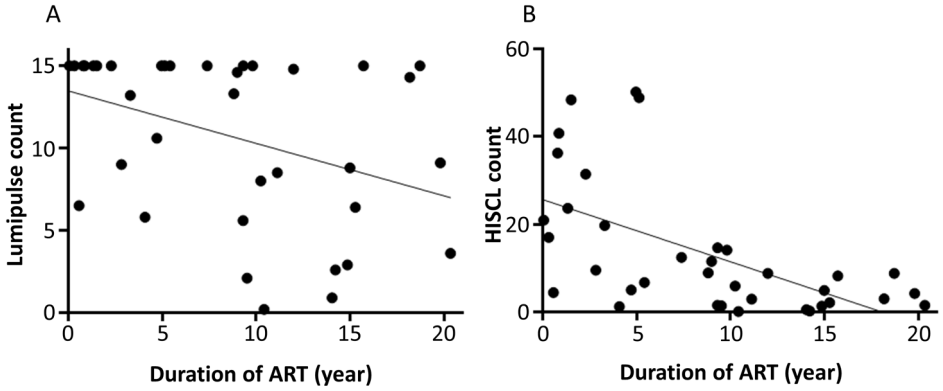

Figure 5. Correlation between duration of ART and (A) Lumipulse S HIVAg/Ab and (B) HISCL™-5000 HIV Ag+Ab using DBS samples. DBS samples were obtained from HIV+ patients on ART. **(A)** 36 DBS samples were analyzed by Lumipulse S HIVAg/Ab. **(B)** 36 DBS samples were analyzed by HISCL™-5000 HIV Ag+Ab.

Table 5. Comparison between DBS and plasma with low count Lumipulse S HIVAg/Ab or HISCL™-5000 HIV Ag+Ab samples

Sample	Sample status	DBS		Plasma	
		Lumipulse count	HISCL count	Lumipulse count	HISCL count
AC	on ART	0.2	0.1	8.7	33.6
AD	acute infection	0.8	0.3	11.9	20.8
AE	acute infection	4.7	0.7	15.0	44.1
AF	on ART	0.9	0.5	15.0	52.8
AG	on ART	2.6	0.2	15.0	43.6

ART: anti-retroviral treatment, DBS: dried blood spot.

absence of several bands, including anti-GP41 antibody, which is one of the targets of LUM.

Among 50 HIV+ donors, 36 were on ART. Next, we investigated the relationship between the duration of ART and LUM or HIS counts. The results showed a significant negative correlation with LUM count (Spearman's correlation factor: -0.430, $p = 0.009$) and HIS count (-0.620, $p < 0.001$) (Figure 5). Finally, we compared LUM and HIS counts of DBS and plasma samples obtained from patients with either LUM or HIS count of < 1.0 from DBS samples (Table 5). Although LUM and/or HIS counts of the DBS were below 1.0, those from plasma were high enough to consider the samples HIV-positive. These results clearly demonstrated the low sensitivity of DBS samples compared to those of plasma samples.

Discussion

The aim of this study was to validate HIV testing with DBS samples derived from self-finger-prick and mailed *via* postal service. The main finding of the study was that the use of DBS is feasible for the screening of HIV infection, and that mailing *via* postal service self-collected DBS to the laboratory does not jeopardize the test reliability.

In this study, it is true that the use of DBS resulted in misdiagnosis of one HIV+ case whereas all cases were diagnosed correctly using the plasma samples. However, 36 of our HIV+ patients were on ART and their viral loads were undetectable. Although our analysis of the relationship between antibody titer and duration of ART was based on a limited number of patients, the results demonstrated a clear correlation between decrease in antibody titers and length of ART use. Other studies also reported low titers of anti-HIV antibodies in patients receiving ART (*12,13*). Thus, it was recommended that data of HIV+ patients on ART should be excluded from evaluation of sensitivity and specificity in HIV testing (*14*). This is one of the limitations of our study. Thus, we consider that HIV+ patients on ART should not be tested by DBS (*3*) in order to avoid misdiagnosis.

Another important aspect of our study is the use of a low cut-off value of 0.5. To elute HIV antigen/antibody from the punched-out DBS containing 8 μL of whole blood (corresponding to 4 μL of plasma), we suspended DBS into 600 μL of PBS and used 100 μL of the supernatant for HIV testing (equivalent to 0.67 μL of plasma). Standard HIV testing requires 100 μL of plasma samples, *i.e.*, 150 times dilution. Our titration study indicated that the LUM count of HIV testing with DBS was 237-times less compared with that of plasma. In our analysis of 50 HIV+ samples, the LUM counts of 3 samples (0.2, 0.8, and 0.9) and HIS counts of 5 samples (0.1, 0.2, 0.3, 0.5. and 0.7) were actually less than 1.0. Therefore, we tentatively lowered the cut-off value to 0.5. Based on additional analysis, we were able to estimate the appropriate cut-off value for DBS HIV testing. Based on the information supplied by the manufacturer, the actual sample volume used in each analysis is 100 μL by LUM and 30 μL by HIS, and the upper limit of LUM count is 15 and HIS 100, indicating that LUM seems to focus on the lower count. We believe that LUM could be better than HIS when the HIV testing system is applied using the DBS samples. Also, we used only two commercially available HIV testing systems, which is another limitation of our study, thus a larger study is needed to select the best system for the DBS HIV testing.

According to the manufacturer, the FTA™ DMPK cards are designed for analysis of drug metabolism and pharmacokinetics. The recommended procedure includes elution of DBS with methanol. In this study, DBS was eluted with PBS, which could have caused inefficient elution from the FTA™ DMPK-B Card based on its low LUM count. Although the FTA™ Classic Card and NucleoCard are intended to preserve nucleic acids, these filter papers seem to perform well with our method. In this study, we adopted Filter paper for blood collection (EIKEN CHEMICAL). The performance of this paper is equivalent to that of 903 Protein Saver Card, which had been used previously in many studies on HIV testing (*5-11*).

In a previous study, we examined the usefulness of DBS collected from MSM and mailed *via* postal service to the laboratory for HIV testing (*15*). The results demonstrated that the time between DBS collection and HIV testing was around 1 week (*15*). In the present study, the between-run experiment confirmed that LUM counts of samples stored at room temperature were relatively stable at least up to 28 days. Therefore, we believe that DBS tested by LUM is potentially suitable for mailed *via* postal service samples collected by self-finger-prick and that such a protocol could be a useful alternative HIV testing system.

In conclusion, the sensitivity of HIV testing with DBS was slightly lower compared to that of plasma. Therefore, DBS is not a replacement for plasma in standard HIV testing. In our study, a single unusual HIV+ case was misdiagnosed by the DBS/LUM system. Nonetheless, HIV testing with DBS can be potentially useful as a screening test in the situation of self-finger-prick and mailing *via* postal service. Applying that, people can receive HIV testing without visiting public health centers which will help in informing them of their HIV status easily and early.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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Approach of Medical Excellence JAPAN to create platforms of collaboration in Asia

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Abstract: Medical Excellence JAPAN (MEJ) is a general incorporated association established in 2011 in Japan. It aims to serve as a central hub and a platform to promote international health business jointly with governments, medical communities, academic organizations, and healthcare industries. This article introduces the works of MEJ in the broader context of Japan Revitalization Strategy. The Act on Promotion of Healthcare Policy (2014 Act No. 48) established the Headquarters for Healthcare Policy, chaired by the Prime Minister and supported by dedicated secretariats in the Cabinet Office. The Headquarters aimed at policy coordination across ministries but learned hard lessons from COVID-19, such as delay of domestic vaccine production. This highlights our systematic weakness of the trajectory from R&D to public availability, and this is the field in which MEJ can play further roles. The value and feasibility of developing MEJ-like mechanisms in Asia with a rapidly growing healthcare sector is discussed.

***Keywords*:** healthcare business, Medical Excellence JAPAN, Asia Health and Wellbeing Initiatives, international collaboration

Introduction

The COVID-19 pandemic has not shown signs of abating since its start in January 2020. More than 186 million people have been infected worldwide, and nearly four million have lost their lives (*1*). This pandemic of "once-in-a-life-time" scale brings large implications to every corner of the globe. Japan is not exempted. Although the impact in Japan caused by the pandemic is relatively small in terms of mortality and morbidity, our over-reliance on foreign production of essential health goods such as surgical masks created consumer panics in March 2020. The insufficient decade-long investment in vaccines left Japan in a situation entirely dependent on vaccines from foreign manufacturers. The pandemic (a health problem) threatens to trigger an economic crisis and even pose a national security challenge (*2*). In the globalized world, countries are interconnected, and people move rapidly on a massive scale. The final report of the Independent Panel for Pandemic Preparedness warns that new pathogens with pandemic potential could emerge at any time. The Panel urges us to take COVID-19 as a wake-up call to move jointly and robustly to make COVID-19 the last pandemic (*3*). How can we take advantage from globalization and mitigate the negative impact of future global challenges? We believe we need to strengthen international collaboration to solve domestic and common international challenges while expanding business opportunities simultaneously.

Medical Excellence JAPAN (MEJ) is a general incorporated association established in 2011 to serve as the central organizational hub to facilitate overseas expansion in the health/medical care sector (*4*). MEJ promotes international health cooperation jointly with governments, medical communities, academic organizations, and healthcare industries. MEJ also provides a business development platform in response to the needs of collaborating countries. In this article, the authors review the past and present works of MEJ and propose the next step to create platforms of collaboration in the post-COVID-19 era.

Concept and creation of MEJ

Japan suffered from economic stagnation caused by the asset price bubble's collapse in late 1991, and economic revitalization has been the top national agenda for many years. Japan has strong research capability,

universal access to quality healthcare services, high-morale personnel, and industries such as pharmaceutical companies that ranked third in originating country of top-selling drugs in the world (*5*). On the other hand, low economic growth and population ageing result in high dependence of Japan's medical expenditures on public resources (*6*). From the international perspective, healthcare markets in neighboring economies are growing at a higher rate than Japan. Given these conditions, international collaboration especially with Asian countries would yield a triple-win situation; specifically, improving health in other countries, achieving business development in Japan, and consequently contributing to sustainability of Japan's social security system.

However, it has proven difficult to create synergy that allows the fragmented actors to work together for more efficient and effective health services delivery, R&D, regulation, production, national economy, and health diplomacy, which are all interlinked. For example, the perspectives and mindsets of the major actors and relevant ministries are diversely different. Healthcare providers and their professional associations prioritize patients and their families at the community level, whereas health-related industries are primarily concerned about shareholder interests. Ministry of Health, Labor and Welfare (MHLW) aims to protect the people's health through stringent regulations. Ministry of Economy, Trade, and Industry (METI) emphasizes industrial development. Ministry of Education, Culture, Sports, Science and Technology (MEXT) focuses on R&D for advancement of science. Ministry of Foreign Affairs (MOFA) and Japan International Cooperation Agency (JICA) address health as a part of development problems in low-income countries. Such diversities make it challenging to set a common agenda for action. However, the other side of the coin is that different stakeholders' self-defined positioning may bring unexpected leverage if mutual complementarity among stakeholders can be achieved.

With the above background, a concept emerged to create at the Cabinet level (1) a platform of "pragmatic" collaboration among key players and (2) a central coordination body across the ministries. Creation of a platform was realized by establishing the MEJ in 2011. This initiative was further activated by the second Abe Cabinet (from December 26, 2012) that emphasized healthcare as one of the Japan Revitalization Strategies (*7*). The mission of the MEJ is shown in Table 1. The initial works were concentrated around the promotion of acceptance of foreign patients by domestic medical institutions (inbound activities) and support of business development of Japanese medical care and related industries overseas (outbound activities).

Regarding strategic coordination at the Cabinet level for seamless stimulations, from R&D to practical application of innovations, products and health systems domestically and internationally, the existing mechanism was reorganized and strengthened by the Act on Promotion of Healthcare Policy (2014 Act No. 48) [unofficial translation under the title "Act to Advance Health and Medicine Strategy" (*8*)]. The Act established the Headquarters for Healthcare Policy, chaired by the Prime Minister, and supported by dedicated secretariats in the Cabinet Office. The Headquarters' official briefing (*9*) listed four specific works including "measures related to the promotion of creation and overseas expansion of new industry activities related to healthcare and medical care" as well as its organizational structure (*10*). This Act together with the Act on Japan Agency for Medical Research and Development (AMED) (*11*) were designed to enhance our research and, consequently, industrial competitiveness under the broader Japan Revitalization Strategy. The Headquarters approved the Healthcare Policy (*12*), which was adopted in 2014 and updated in 2017, 2019 and 2021, as well as the Plan for Promotion of Medical Research and Development (*13*) adopted and updated together with the Policy. The Headquarters also approved the Basic Principles of the Asia Health and Wellbeing Initiative (AHWIN) in 2016 (revised in 2018) (*14*). Under this initiative, the exchange of long-term care-related personnel and collaboration of long-term care services with overseas countries have expanded.

Table 1. Mission statement of MEJ

Medical Excellence JAPAN (MEJ) aims to contribute to the health, social welfare and economic improvement of people around the world through the provision of medical services and technologies provided through cooperation between the governments of Japan and other nations as well as these nations' respective medical and business/industrial communities.

● Contributing to Global Healthcare
MEJ aims to contribute to the development and improvement of medical services around the world through the provision of medical services and technologies appropriate for the characteristics of each individual country and region.

● Contributing to Health Care in Japan
MEJ aims to contribute to the development of medical services in Japan by supporting improvements in Japan's capacity to provide international medical services.

● Contributing to Economic Development
MEJ plans to contribute to economic growth in Japan around the world by supporting the growth of medical institutions and medical-related industries.

Consequently, business activities in related fields are becoming active. Also, the Basic Principles of the Africa Health and Wellbeing Initiative (*15*) were adopted in 2019.

Various initiatives and lessons of vaccine defeat

As discussed above, under the Abe Cabinet, attempts to enhance coordination among ministries made progress. However, these organizational changes did not bring a substantial outcome in Japan's response to the COVID-19 pandemic. It is unfortunate that we did not observe the warp speed R&D, clinical trials, approval, production, and access for COVID-19 vaccine. Various systems have policy objectives reflecting the originating ministries, and the barrier was difficult to overcome. Attempting to visualize the involvement of different ministries and providers in each process of pharmaceutical R&D, the authors have constructed a matrix as shown in Figure 1.

There are various steps before medical products can become widely accessible. The first step is to find the seed compound through basic research. Then the safety and effectiveness of the compound are verified. This is followed by approval through various regulatory procedures. After the developer's intellectual property is protected, the product is produced and marketed based on business strategies according to market demand such as price setting. In reality, these steps may proceed in parallel.

Next, the above steps will be examined more carefully regarding the various arrangements provided by different sectors. From the health sector, AMED offers basic and applied research funding, and MHLW provides a legal framework and incentives for promotion of domestic and overseas clinical trials. To encourage innovative medical products uniquely from Japan, a special measure for regulatory approval called the Sakigake Designation System (*16*) is provided by the Pharmaceuticals and Medical Devices Agency (PMDA). In addition to quicker approval, indirect support is provided by the investment of the Japanese Government in Gavi (vaccine), Global Fund (AIDS, tuberculosis, malaria drugs, and diagnostics), and UNITAID (AIDS, tuberculosis, malaria, hepatitis drugs, and diagnostics) that support procurement of medical products for low income countries. However, the middle part of the process; business and production, is handled by companies and manufacturers. This part could be "the valley of death".

Also, it is worth reviewing other support schemes provided by ministries and MEJ in development of international health business. MOFA and JICA support local activities involving the initial project development in collaborating countries, such as private sector cooperation projects and model projects, through technical cooperation. To strengthen collaboration with low and middle-income countries, they offer assistance in developing relevant infrastructure through grants-in-aid, loans, and technical cooperation. This surely enhances the absorption capacity of new products and technologies.

METI supports the stage of project formation, and a typical project is "Development of International Healthcare Hubs" (*17*). Also, METI in collaboration

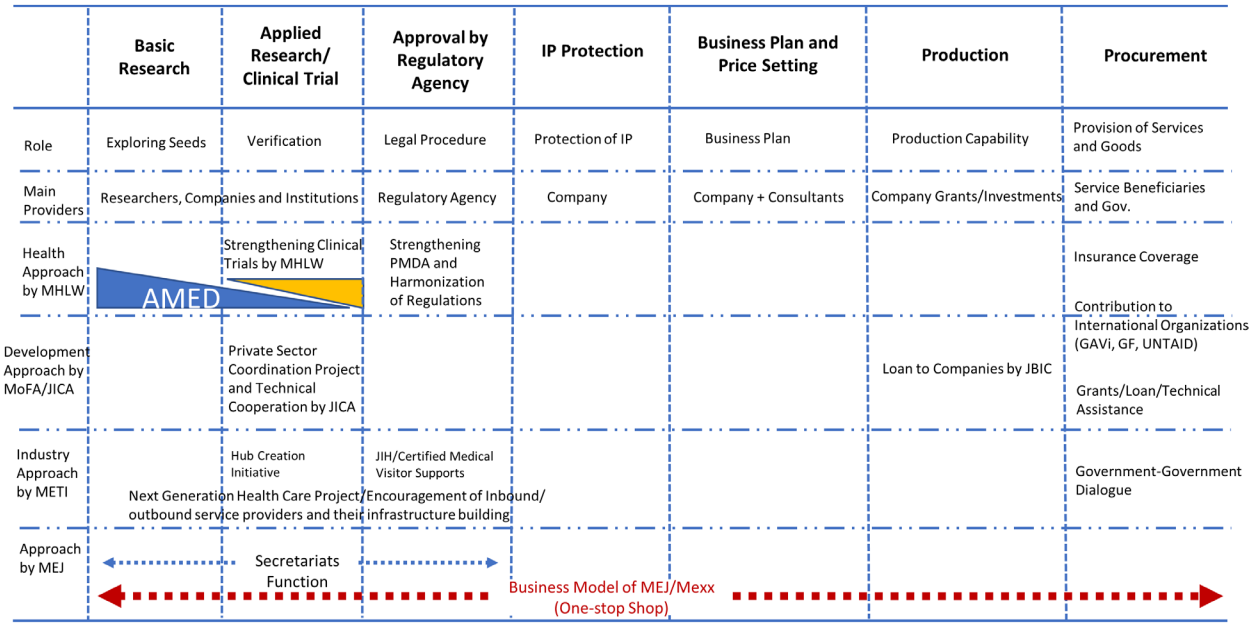

Figure 1. Mapping of broadening international health care products and business at various stages of pharmaceutical research and development.

with Japan External Trade Organization (JETRO) and MEJ supports the development and promotion of new services, service providers, enlightenment, training, and infrastructure development for next-generation medical/inbound/outbound businesses. The Japan Bank for International Cooperation (JBIC) provides loans to overseas projects undertaken by Japanese companies willing to expand overseas.

Policy dialogues are conducted between governments, resulting in MOUs with countries such as India and Vietnam. However, there is a wide perception that these mechanisms are not optimally functioning in developing the COVID-19 vaccines in Japan and some perceive this as the "lost war in vaccine competition". Meanwhile, the world is moving towards preparing for the next pandemic, as symbolized by the G7 summit and G7 Health Ministers' agreement in boosting international clinical trials to address the bottleneck of evaluating the efficacy and safety of the needed medical products (*18*).

What is the position of MEJ? As shown in Figure 1, MEJ is currently serving the function as secretariat and the role as the executive agency of the METI industrial policy approach, which is indicated by the red dotted line in the figure. On the other hand, for any business entity, it is undoubtedly desirable to have one-stop support and advice during the entire R&D process from seed-finding to getting on track with business. Until now, support options are concentrated in the upstream part of the flow. However, it will be necessary to provide assistance for the middle part; business and production, where many business entities struggle. This is the part where accumulated experience and knowledge are unfortunately not shared effectively. In the future, MEJ should make a business plan on how to overcome this "valley of death" and to develop activities by obtaining funds in the form of sustainable investments from corporate business entities.

Future development

Medical care in Asia is expected to grow in volume and in quality through market expansion and mutual exchange. New efforts are required to further promote the international business development of Japanese-style medical care. In this respect, the vision of MEJ is to speedily introduce a "patient-centered rational medicine initiative" to like-minded countries, to substantiate this initiative in absolute terms to improve medical care quality, and to create a new mutually beneficial arrangement for health security through international medical care.

At the same time, as shown in ASEAN Economic Community Blueprint 2025 (*19*), cooperation in the medical field is progressing steadily in Asia. To take advantage of such momentum in ASEAN countries, we need an environment in which industry, government, academia, and health care providers can discuss on the same platform. As mentioned above, in industry-government-academia-medicine collaboration, each ministry has its own unique policy objectives. The perspectives, mindsets, interests, and priorities of major stakeholders are diverse. Therefore, comprehensive coordination is essential and is a common challenge for both Japan and other Asian countries. One possible measure to alleviate these bottlenecks is to create MEJ-like forums (tentative name, MExx) in countries with large market sizes, such as India, Vietnam, the Philippines, and Indonesia. In the future, when these forums collaborate mutually at each platform, health improvement and competitiveness of the Asian health care industry can be expected to improve further. With this background, the Economic Research Institute for ASEAN and East Asia (ERIA) (*20*) has set up a secretariat for coordination, and full-scale activities will start from the latter half of 2021. The MEJ aims to facilitate this next generation of collaboration for health and economic prosperity for all countries involved.

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Intraoperative indocyanine green fluorescence navigation facilitates complete removal of lymph node metastases from hepatocellular carcinoma

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Abstract: Indocyanine green (ICG) is a fluorescent dye that selectively accumulates in primary hepatocellular carcinoma (HCC) as well as in extrahepatic metastases of HCC. Reported here is a case of metachronous lymph node (LN) metastases from HCC that were resected using ICG fluorescence navigation. A man in his 70s was referred to this department for suspected LN metastasis from HCC. Computed tomography revealed an enlarged suprapancreatic LN. After a laparotomy, an ICG fluorescence imaging system intraoperatively revealed strong fluorescence of this LN, which was then easily resected. An examination after the removal of the LN revealed fluorescence from the adjacent lymphatic tissue as well, so an additional resection was performed. Pathologically, both LNs were confirmed to be metastases from HCC. In this case, some lymphatic tissue metastases from HCC could not be identified prior to surgery, but intraoperative use of ICG fluorescence navigation facilitated their complete removal.

***Keywords*:** hepatocellular carcinoma, LN metastasis, indocyanine green, fluorescence navigation surgery

Indocyanine green (ICG) has been used for perfusion diagnosis of tissue and navigation to locate sentinel lymph nodes (LNs) since ICG emits light with a peak wavelength of approximately 800 nm when excited with near-infrared light. Recent studies have indicated that ICG fluorescence imaging was useful at detecting primary HCC and extrahepatic metastases from HCC (*1-3*). Reported here is a case where ICG fluorescent navigation was intraoperatively used to detect LN metastases of HCC that could not be identified prior to surgery.

A man in his 70s was referred to this department for resection of LN metastasis of HCC. He had a history of hepatitis C infection, but a sustained virologic response was achieved with interferon treatment. He had undergone left lateral segmentectomy for HCC 26 years ago and distal gastrectomy for gastric cancer 16 years ago. Transcatheter arterial chemoembolization was performed twice for intrahepatic recurrence in segment VI 12 months ago and in segments IV & VIII 6 months ago. Nevertheless, the serum levels of alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II) gradually increased (AFP: 1751 ng/mL, PIVKA-II: 172 mAU/mL). Thus, further examination was performed. Contrast-enhanced dynamic computed tomography revealed no intrahepatic recurrence and a swollen suprapancreatic LN that had increased in size from 11 mm to 16 mm over the last 3 months (Figure 1A and 1B). This LN was highly enhanced in the arterial phase, which accorded with the metastasis of HCC. Positron emission tomography-computed tomography revealed the accumulation of fluorine-18 deoxyglucose in this LN (Figure 1C). SUVmax was 2.05. There was no evidence of recurrence in any other regions, and resection of this LN was scheduled.

ICG (Diagnogreen, Daiichi Sankyo, Tokyo, Japan) was injected intravenously at a dose of 0.5 mg/kg body weight, 1 day before surgery. After a laparotomy and sharp dissection of severe adhesions, a swollen LN was palpated in the suprapancreatic area. Exploration with a near-infrared light camera system (PINPOINT®, Stryker Corporation, Kalamazoo, MI) revealed strong fluorescence from this LN (Figure 2A). After the resection of this LN, the abdominal cavity was explored with the ICG camera again. The cranial side of the resected area was still emitting fluorescence (Figure 2B), so an additional resection was performed. After fluorescence was no longer observed (Figure 2C), surgery was concluded.

Hematoxylin and eosin staining of resected specimens revealed that the normal LN structure was replaced by atypical cells (Figure 3A). Immunohistochemical analysis indicated that these cells were positive for

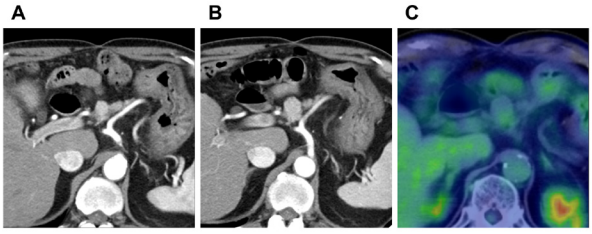

Figure 1. (A) CE-CT revealed a swollen LN 11 mm in diameter with enhancement in the arterial phase (arrow). **(B)** Three months later, CE-CT revealed enlargement of this LN, which was now 16 mm in diameter, with enhancement in the arterial phase (arrow). **(C)** PET-CT revealed FDG accumulation in this LN. CE-CT: contrast-enhanced computed tomography, PET-CT: positron emission tomography-computed tomography, FDG: fluorine-18 deoxyglucose, LN: lymph node.

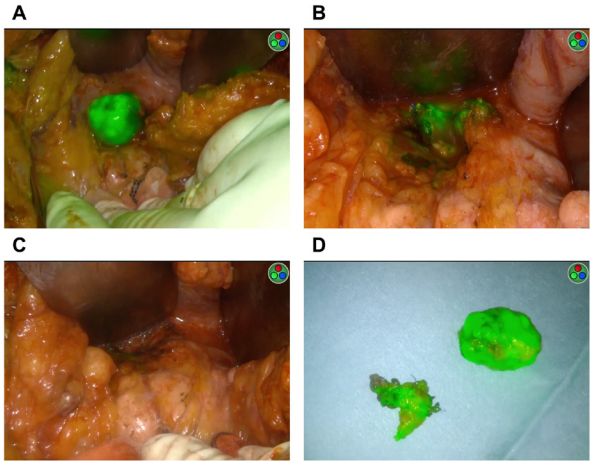

Figure 2. ICG fluorescence imaging using the PINPOINT® camera system. (A) A swollen LN with a strong fluorescent signal. **(B)** After the resection of the swollen LN, the strong fluorescent signal remained. **(C)** After the additional resection, no fluorescent signal was detected. **(D)** ICG fluorescence image of the resected specimens. ICG: Indocyanine green.

Glypican-3 and Hep Par1 and negative for CK7 and CK20 (Figure 3B). Based on these results, the resected LNs were pathologically confirmed to be metastases of HCC. Soon after surgery, elevated tumor markers returned to normal levels (AFP: 33 ng/mL, PIVKA-II: 10 mAU/mL) (Figure 4). No recurrence of HCC has been detected for more than 2 years after surgery.

Extrahepatic recurrence of HCC is relatively rare compared to intrahepatic recurrence. The common sites of extrahepatic metastases are the lungs, bones, and LNs (*4*). LN metastasis is found in 3-8% of patients with HCC. An effective standard treatment for LN metastasis has yet to be established, and the prognosis of LN metastasis is generally poor. As a result of 2 phase III studies (the SHARP trial and the Asia Pacific trial), sorafenib has been regarded as the standard treatment for advanced (unresectable or metastatic) HCC (*5,6*). However, sorafenib has not been found to provide a significant survival benefit according to a subgroup analysis of patients with LN metastasis. Although the clinical significance of surgical resection of LN metastasis

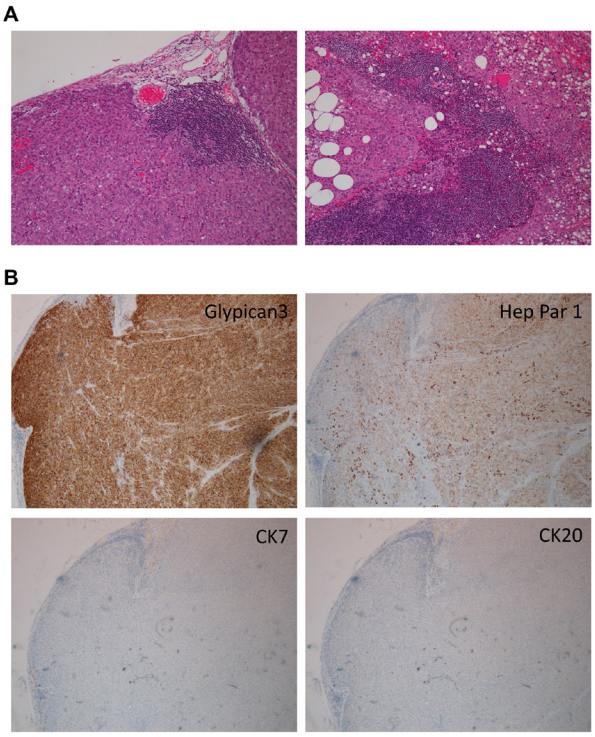

Figure 3. (A) Pathological findings for the resected LNs: both LNs contained metastatic hepatocellular carcinoma cells (left; swollen LN, right; additional resected LN, hematoxylin and eosin staining, ×100). **(B)** Immunohistochemistry of the resected LN (×40). The LN was positive for Glypican-3 and Hep Par 1 and negative for CK7 and CK20 (×40). LN: lymph node.

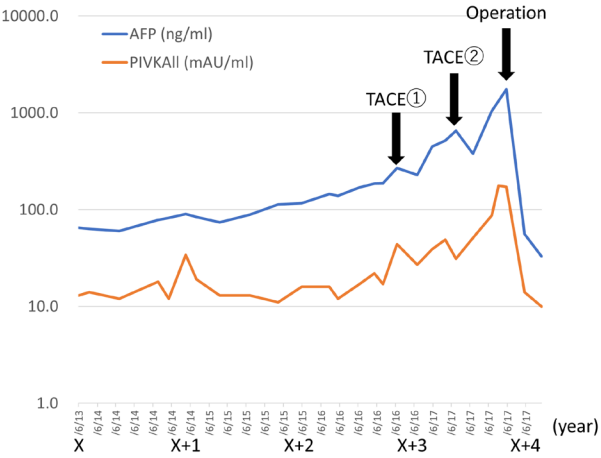

Figure 4. Changes in serum AFP and PIVKA-II levels. Serum AFP and PIVKA-II levels rapidly decreased within the normal range. AFP: alpha-fetoprotein, PIVKA-II: protein induced by vitamin K absence-II.

remains controversial, some studies have reported that the resection of LN metastasis was associated with long-term survival, and especially in patients with resectable or controllable intrahepatic lesions (*7,8*). In the current case, no other recurrent lesions were detected, so complete resection of LN metastasis should allow a longer survival.

ICG is a fluorescent dye that emits light with a peak

wavelength of approximately 800 nm when excited with near-infrared light and was initially used to evaluate liver function and cardiac output. As real-time fluorescent imaging systems have developed, ICG has been used for perfusion diagnosis of tissue and navigation to locate sentinel LNs. In addition, recent studies have revealed that ICG fluorescence imaging was useful at detecting primary HCC and extrahepatic metastases from HCC because of the selective uptake and the prolonged retention of ICG (*1-3,9*).

An accurate diagnosis of LN metastases is essential for determination of the appropriate surgical procedure. However, preoperative imaging studies using modalities such as CT and MRI assess LN metastases based on criteria like size and shape. In patients with HCC, hilar or peripancreatic LNs are often swollen because of accompanying liver inflammation. Therefore, distinguishing between swelling of benign LNs and malignant LNs is difficult. Moreover, determining whether a small LN is metastatic or not is even more difficult. Since multimodal diagnosis for the N-staging of gastric cancer has a reported accuracy of only 50-90% (*10*), preoperative diagnosis of LN metastasis from HCC presents a challenge.

Hence, more sensitive and specific methods of diagnosis should be developed. Satou *et al*. reported that 14 (93%) out of 15 LN metastases from HCC were detectable with ICG fluorescent imaging (*3*). In the current case, lymphatic metastases that were not evident prior to surgery were identified. Although ICG fluorescence imaging has a drawback in that it cannot detect fluorescence from a deep lesion, it could be a useful tool with which to detect LN metastases.

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Benefits of physical therapy for people living with hemophilia

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Abstract: This crossover study investigated effects of physical therapy (PT) on motor function in patients with hemophilia infected with human immunodeficiency virus (HIV) due to treatment with non-heat-treated blood products. Patients were randomly divided into a PT-first group (PT once monthly for 6 months, then only home exercise (HE) for 6 months) and an HE-first group (HE for 6 months, then PT once monthly for 6 months). Carryover, period, and treatment effects were examined. Carryover effect was observed for flexion muscle strength of the right hip joint and fast walking cadence. Period effect was observed for extension Range of motion (ROM) of the left hip joint, dorsiflexion of the right ankle joint, and fast walking cadence. PT tended to be effective for abduction of the left shoulder joint and fast walking cadence. Compared with HE, PT tended to contribute to improving ROM of the shoulder joints and fast walking.

***Keywords*:** home exercise, crossover, cadence, walking

Physical therapy programs for persons with hemophilia were introduced in the Southern California area in 1959 (*1*). By the 1960s, the importance of exercise therapy was demonstrated, with the effects of preventing joint deformity and bleeding, and relieving pain (*1,2*). However, patients with hemophilia do not have much experience with exercising, and many do not perform daily active physical exercise (*3*).

In a study by Goto *et al*. (*3,4*), the effects of home exercise were examined and demonstrated to improve physical function and activities of daily living (ADL), and to prevent bleeding. Also, in terms of the necessity and effects of physical therapy, Wittmeier (*5*) showed that physical therapy helped in prevention and recovery from bleeding and improved levels of physical activity. Forsyth *et al*. (*6*) also showed that in order for hemophilia patients to start exercising, physical therapists who can properly evaluate the exercise are indispensable. Therefore, in the present study, we investigated the effects of physical therapy in patients with hemophilia infected with human immunodeficiency virus (HIV) due to treatment with non-heat-treated blood products, by comparing the effects of home exercise with versus without once-monthly physical therapy delivered by a physical therapist for 6 months.

This study was conducted at the Department of Rehabilitation Medicine, National Center for Global Health and Medicine between October 2015 and November 2017. Inclusion criteria were ability to undergo evaluation of joint range of motion, muscle strength, grip strength, and 10-m walking, and provision of informed consent to participate in the study. Ability to undergo the evaluation of 10-m walking was not an essential requirement. Exclusion criteria were hemarthrosis, intraarticular bleeding, intramuscular bleeding, and unhealed fractures on the day of evaluation.

Participants were divided into 2 groups by the envelope method, and an unblinded crossover study was conducted. One group was the physical therapy-first group, and the other was the home exercise-first group. Both groups underwent initial evaluation. After that, the physical therapy-first group received physical therapy once monthly in the first 6 months (Period I), performed home exercise using an instruction leaflet without monthly interventions by a physical therapist in the following 6 months (Period II), and then underwent final evaluation. The home exercise-first group performed home exercise in Period I, received physical therapy once monthly in Period II, and then underwent final evaluation. Home exercise and physical therapy were performed in the same way in the home exercise-first group as in the physical therapy-first group.

Evaluation items were joint range of motion (flexion and abduction of the shoulder joint; flexion, extension, pronation, and supination of the elbow joint; flexion, extension, and abduction of the hip joint; straight leg raising (SLR); flexion and extension of the knee joint; flexion and dorsiflexion of the ankle), muscle strength

(flexion, extension, and abduction of the hip joint; extension of the knee joint), and 10-m walking (normal and fast walking). Muscle strength was evaluated using a handheld dynamometer μTasF-1 (Anima Corp., Tokyo, Japan) using the method developed by Hirasawa *et al*. (*7*). In 10-m walking, participants were instructed to walk at 2 speeds, normal and fast walking, and the speed, cadence, and stride length were analyzed.

Physical therapy included *i*) confirmation of the participant's status at 1 month (*i.e*., status of activity, bleeding and pain, and exercise); *ii*) confirmation and correction of stretch movements conducted at home; *iii*) range of motion training on several joints, depending on the evaluation result, by a physical therapist; *iv*) confirmation and correction of the form of muscle strength training conducted at home; *v*) guidance on changing the content of muscle strength training conducted at home (exercise menu and loads); and *vi*) confirmation and guidance on walking, and ascending and descending stairs.

Regarding home exercise, in accordance with the instruction leaflet, which was developed for patients with hemophilia (*8*), an exercise menu was prepared for each participant based on the results of motor function evaluation.

Differences in age, body mass index, range of motion, muscle strength, and walking ability at the start of the study between the physical therapy-first group and the home exercise-first group were examined using unpaired *t*-test. The crossover results were assessed using the *t*-test for period effect, treatment effect, and carryover effect on each evaluation items as described previously (9). Briefly, carryover effect was tested on the Period (I+II) data in both groups; treatment effect on Period (I-II)/2 data in both groups; and period effect on Period (I-II)/2 data in the physical therapy-first group, while on Period (II-I)/2 data in the home exercise-first group. Analyses were performed using SPSS ver. 26 (IBM, Armonk, NY, USA).

This study was approved for central review by the Ethics Review Committee of the National Center for Global Health and Medicine (approval number, NCGM-G-003242-00), and appropriate ethics procedures were followed at each participating facility.

After excluding participants who dropped out of the study, the remaining participants were all men: 11 in the physical therapy-first group (mean age 51.9 years, mean height 171.83 cm, mean weight 61.87 kg) and 7 in the home exercise-first group (mean age 53.3 years, mean height 175 cm, mean weight 59 kg). There was no difference in age or BMI between the groups.

The following items were excluded from the analysis because the baseline measurements were significantly higher in the physical therapy-first group than in the home exercise-first group: flexion range of motion of the left shoulder joint, abduction range of motion of both hip joints, right SLR, and left SLR.

Table 1. The *p*-values for carryover effect, treatment effect, and period effect

Range of motion	Carryover effect	Treatment effect	Period effect
Right shoulder joint			
Flexion	0.195	0.147	0.421
Abduction	0.773	0.444	0.172
Left shoulder joint			
Abduction	0.540	0.052	0.182
Right elbow joint			
Flexion	0.872	0.728	0.324
Extension	0.142	0.983	0.356
Pronation	0.848	0.638	0.532
Supination	0.547	0.126	0.932
Left elbow joint			
Flexion	0.962	0.923	0.086
Extension	0.245	0.605	0.155
Pronation	0.468	0.488	0.488
Supination	0.582	0.468	0.107
Right hip joint			
Flexion	0.116	0.511	0.511
Extension	0.074	0.529	0.056
Left hip joint			
Flexion	0.78	0.188	0.067
Extension	0.592	0.978	0.023*
Right knee joint			
Flexion	0.135	0.808	0.223
Extension	0.204	0.668	0.267
Left knee joint			
Flexion	0.912	0.275	0.886
Extension	0.586	0.505	0.685
Right ankle joint			
Dorsiflexion	0.825	0.33	0.003*
Plantar flexion	0.804	0.144	0.601
Left ankle joint			
Dorsiflexion	0.167	0.814	0.31
Plantar flexion	0.593	0.694	0.098
Muscle strength	**Carryover effect**	**Treatment effect**	**Period effect**
Right hip joint			
Flexion	0.009*	0.691	0.368
Extension	0.146	0.782	0.056
Abduction	0.13	0.477	0.428
Left hip joint			
Flexion	0.144	0.666	0.565
Extension	0.279	0.231	0.87
Abduction	0.385	0.403	0.506
Right knee joint			
Extension	0.291	0.522	0.244
Left knee joint			
Extension	0.973	0.199	0.136
Normal walking			
Walking speed	0.106	0.811	0.138
Stride length	0.434	0.469	0.769
Walking cadence	0.327	0.447	0.816
Fast walking			
Walking speed	0.108	0.364	0.534
Stride length	0.329	0.636	0.117
Walking cadence	0.092*	0.091	0.009*

*Asterisks denote statistical significance. A *p*-value of 0.1 was considered significant for carryover effect, while that of 0.05 for others.

Table 1 shows *p*-values for carryover effect, treatment effect, and period effect on range of motion, muscle strength and walking ability. Carryover effect was observed for flexion muscle strength of the right hip joint and cadence in fast walking. Period effect was observed for extension range of motion of the left hip joint, dorsiflexion range of motion of the right ankle joint, and cadence in fast walking. A significant

treatment effect was not observed, but physical therapy tended to be effective for abduction range of motion of the left shoulder joint and cadence in fast walking.

In patients with hemophilia, hemarthrosis is accompanied by synovitis and arthropathy, resulting in muscle atrophy around the joint. Tiktinsky *et al*. (10) pointed out that excessive rest after bleeding causes muscle atrophy and joint contracture, resulting in a vicious cycle of rebleeding and impairment in ADL. To break this vicious cycle, physical therapy and exercise are recommended for patients with hemophilia (*1,2,11-14*).

Tiktinsky *et al*. (*10*) demonstrated that continuous resistance training over 1 to 2 years resulted in increased muscle strength around the knees and elbows, prevention of bleeding, and pain relief in 2 adult patients with severe hemophilia. Hilberg *et al*. (*15*) reported that a 6-month-long physical training program in 9 adult patients with severe hemophilia resulted in a 34% increase in lower limb muscle strength and an improvement in proprioception and coordination. Goto *et al*. (*4*) examined the effects of 8-week home exercise in 32 adult patients with hemophilia. Effects of home exercise were compared between 2 groups: one group simply performed exercises and the other performed exercises that incorporated self-monitoring. Results showed no difference in range of motion, balance ability, and walking speed, regardless of whether exercises incorporated self-monitoring. As described above, there are some reports on exercise therapy in patients with hemophilia; however, to our knowledge, no study to date has compared the effects of physical therapy with the effects of home exercise. In 2017, Ruben *et al*. (*16*) examined the effects of educational intervention together with home exercise conducted over 15 weeks in patients with hemophilia. However, the evaluation items were pain and quality of life, and motor function was not examined in detail. The outpatient physical therapy in the present study was not limited to manual training of range of motion or muscle strengthening, and each session also included evaluation of motor function and instruction on self-training based on the evaluation results for each patient.

A washout period was not included in this study, but a carryover effect was observed for only flexion muscle strength of the right hip joint and cadence in fast walking. On the other hand, a period effect was observed for extension range of motion of the left hip, dorsiflexion range of motion of the right ankle, and cadence in fast walking, suggesting that left hip extension and right ankle dorsiflexion worsens and cadence in fast walking improves over time. The treatment effect on fast walking was not significant, but showed a tendency toward improvement with physical therapy ($p = 0.091$).

Fast walking ability is a practical requirement in daily living (*17*). Goto et al. demonstrated improvement in walking, but whether the walking speed was normal or fast was not described (*3*). The present study examined both normal walking and fast walking, and found improvement in cadence in fast walking.

The exercise menu in this study was designed for each patient based on the results of motor function evaluation. Use of such personalized intervention in this study showed a tendency toward improvement of walking at practically important speed, which was not reported previously. This suggests that accurate evaluation and provision of instruction and a self-training exercise menu based on the evaluation results, rather than simply providing range of motion training or muscle strengthening training manually, may be more effective in physical therapy to improve motor function in patients with hemophilia and HIV infection.

One of the limitations in this study was that the way patients performed home exercise was not followed in detail, and their adherence to our instruction was judged based on their verbal reports only. Greene et al. reported that it is difficult to achieve favorable adherence to home exercise in patients with hemophilia due to their fear of bleeding (*18*). A method for accurately monitoring the performance of home exercise is needed in order to provide more effective instructions in the future. Another limitation is that a washout period was not included in this study. Consequently, a carryover effect on results in Period II was observed, albeit only for a few measurement items. To validate the effect of physical therapy in the future, comparisons should be performed in studies with an adequate washout period.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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Algeria's preparedness for Omicron variant and for the fourth wave of COVID-19

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Abstract: The world has been confronting a health crisis for two years because of the COVID-19 pandemic. The context of the crisis has the form of the first wave and subsequent waves that varied depending on the country. Undoubtedly, Algeria is one of the countries that have suffered severely from this pandemic. The recent wave has been marked with the huge number of casualties given the poor preparation. The country's preparation issue will be more complicated. In other words, in the context of an expected 4th wave that is characterized with the appearance of the new Omicron variant B.1.1.529. Thus, this news will consider the situation comprehensively and will provide recommendations to minimize the potential damage that will result from the fourth wave, and to attempt to limit the spread of the virus. The updated measures aim at rising the opportunities of improving the health situation in the country in the near future.

***Keywords*:** COVID-19, pandemic, Algeria, Omicron, global health

Similar to many countries, Algeria has extensively suffered from COVID-19. The first case was reported on February 25, 2020. Early 2021, Algeria has shocked the world by reporting one of the highest fatality rate with approximately 15% (*1*).

So many countries announced officially the fourth wave of COVID-19. In most cases, this wave was quite deadly despite the democratization of vaccination. Concerning Algeria, many specialists predicted a next 4th wave around December 2021. In this regard, a lot of works has recently been published concerning predictive models of cases, deaths or future waves of the virus in the country (*2*). However, in most cases the spread of this virus has often differed from the predictions.

Despite governmental efforts, there are still deficiencies in testing; meaning the number of infections is likely to be an underestimate of actual numbers because they are based on PCRs only.

Thus, the question that arises is: "Is Algeria ready for a 4th wave?"

Firstly, throughout the 3rd wave, the country experienced a rather murderous period by recording 1927 cases and 49 deaths the July 28, 2021 (Figure 1). At that period, the country was already poorly prepared. This is in particular with the appearance of the Delta variant, which caused a lot of damage and led to the emergence of several clusters. The damage was obvious especially in the northern cities as they represent the most densely populated areas. Therefore, the third wave has caused many deaths that could have been avoided specifically in people suffering from comorbidities (*3*).

Further, despite the fact that Algeria has ordered fairly large doses of vaccines (AstraZeneca, Sputnik V, and Sinovac), the vaccination remains quite humble because only 11% of the population is fully vaccinated (*4*). Some surveys have revealed that people do not have a firm confidence in vaccination (*5*), which might justify the vaccine refusal from a large majority of the population.

Fundamentally, facts like the growing lack of severity in the application of preventive measures, the gradual opening of borders will most certainly be unhelpful in dealing with the issue.

In addition to the previous reasons, the appearance of the Omicron variant in South Africa, which is already present in 57 countries according to the World Health Organization (WHO) (*6*), will make the situation firmer and more delicate to treat. The variant was characterized by an impressive diffusion speed, particularly in the neighbouring countries of South Africa (Eswatini, Zimbabwe, Mozambique, Namibia and Lesotho). While currently little information is provided, scientists predict that after the third dose, the variant is unlikely to be more dangerous than the Delta variant which remains dominant. Actually, there has yet been no detection in Algeria, but everything suggests that it is only a matter of time.

In conclusion, to avoid a fourth wave's significant

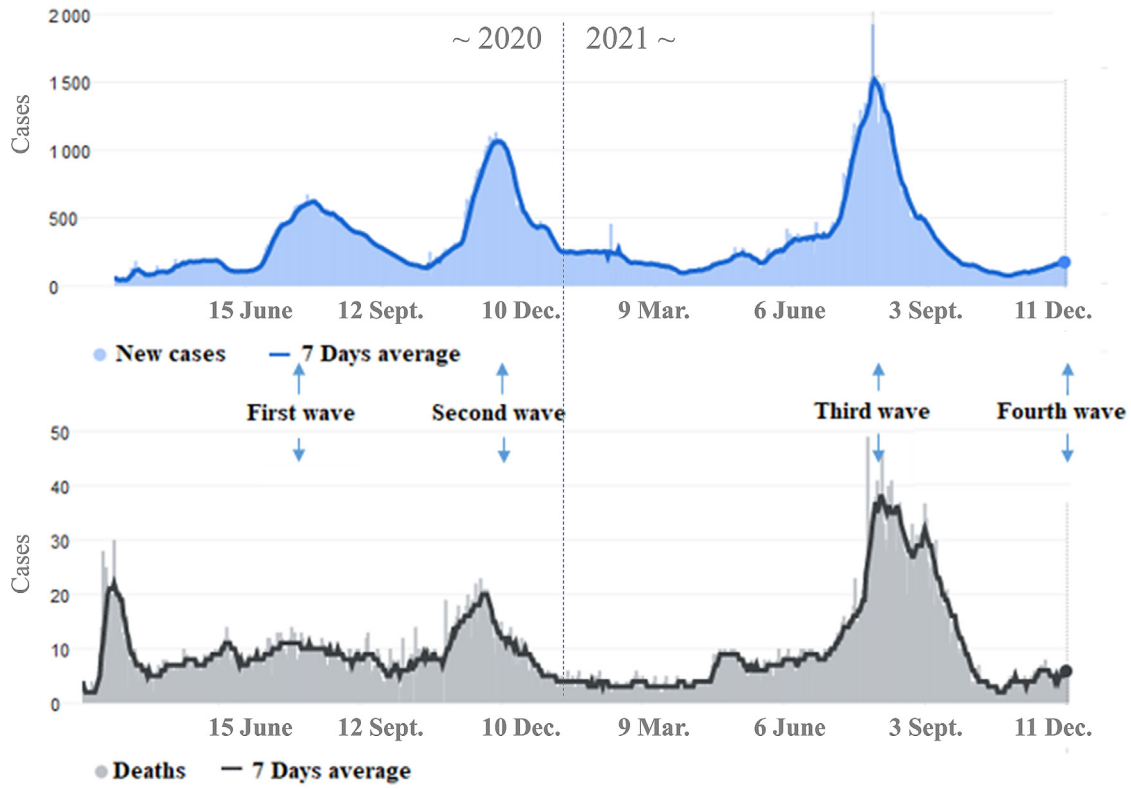

Figure 1. Daily cases and deaths during the three waves of COVID-19 pandemic in Algeria. (Data source: *https://coronavirus.jhu.edu/map.html*).

losses, the continuity of the strict application of protective measures (wearing a mask, washing hands, social distancing, etc.) and making the necessary arrangements for the availability of oxygen are recommended. It is also necessary to perform an intensive vaccination process to optimise protection.

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