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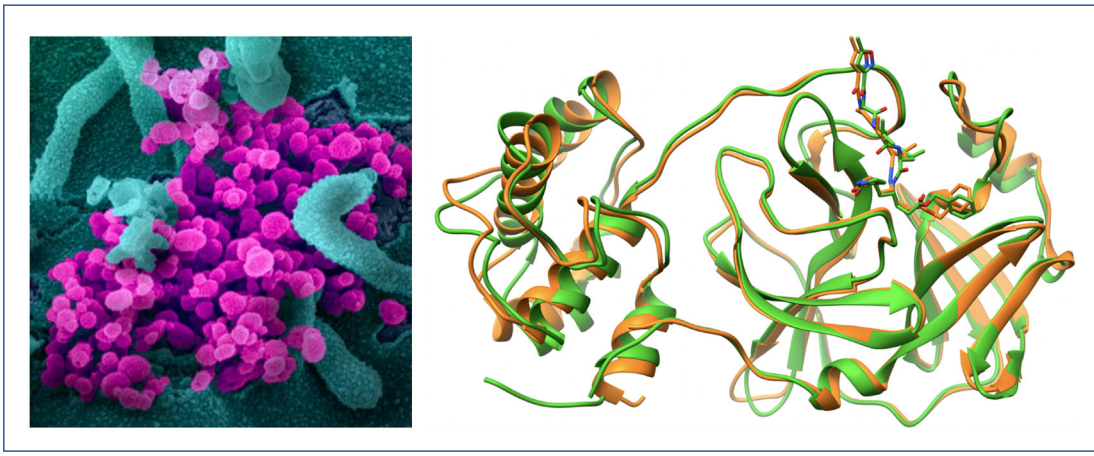

Scanning electron micrograph of SARS-CoV-2 virions (magenta round objects) and superimposition of the main protease of SARS-CoV-2 (green) over the main protease of human coronavirus NL63 (orange)

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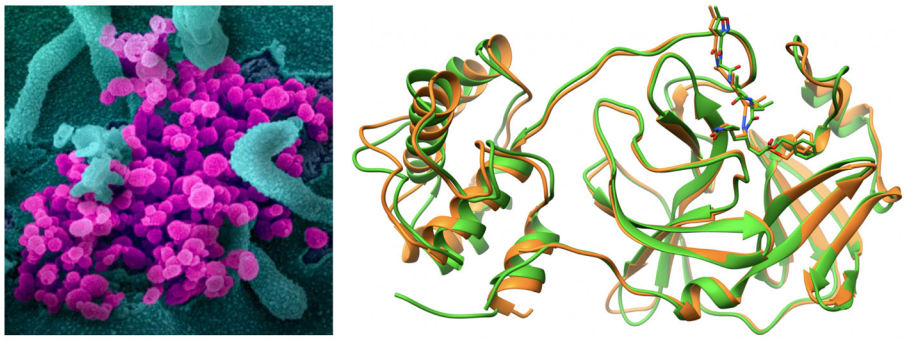
CONTENTS

Volume 2, Number 1, 2020

CONTENTS (Continued)

COMMUNICATION

COVER FIGURE OF THIS ISSUE



Scanning electron micrograph of SARS-CoV-2 virions (magenta round objects; *https://www.nih.gov*) and superimposition of the main protease of SARS-CoV-2 (PDB entry 6LU7; in green) over the main protease of human coronavirus NL63 (5GWY; in orange). Both proteases on the right are in complex with a covalent protease inhibitor N3 and are highly conserved particularly in their catalytic active sites. The main protease of SARS-CoV-2 cleaves viral polyproteins at 11 sites and may serve as an important drug target.

Haydar Bulut and *Hiroaki Mitsuya*

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Emergence of SARS-CoV-2 and its outlook

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Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported at the end of 2019 in China. By the end of February 2020, the virus has spread worldwide through continuous human-to-human transmission via contact and droplet infection, demonstrating the ease with which emerging viruses disperse globally through the mass transport system. Here, we summarize our knowledge of other coronaviruses that have infected humans in comparison with SARS-CoV-2.

***Keywords*:** SARS-CoV-2, human coronaviruses, intermediate host, reservoir

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, provisionally named 2019-nCoV) emerged in Wuhan, Hubei Province, China at the end of 2019 as the seventh human coronavirus (HCoV) (*1*) and spread throughout the world. SARS-CoV-2 causes coronavirus disease 2019 (COVID-19), which can be asymptomatic or cause fever, cough, shortness of breath, acute respiratory distress syndrome (ARDS), pneumonia, and death (*2*).

As of February 27, 2020, the WHO reported that more than 82,000 cases of COVID-19 have been identified in 46 countries/regions of Asia, South and North America, Europe, Africa, and Oceania (*https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/*). In particular, South Korea, Italy, Japan, Iran, and Singapore, together with China, have each reported more than 100 cases of COVID-19.

Most of the recent COVID-19 patients in Japan claim not to have visited China or to have had close contact with COVID-19 patients, indicating that SARS-CoV-2 might have invaded the Japanese population. The National Institute of Infectious Disease (NIID) and local institutes of public health in Japan are using nested RT-PCR and/or real-time RT-PCR for diagnosis, and the NIID together with Japanese companies has initiated the development of a rapid diagnosis kit (based on immunochromatography). The development of vaccines and antivirals against SARS-CoV-2 are also planned in Japan as well as other countries. Furthermore, basic research on SARS-CoV-2 is underway to understand its behavior in natural and intermediate hosts and humans.

SARS-CoV-2, which belongs to lineage B of Betacoronavirus within the order Nidovirales and family Coronaviridae, possesses a single positive-strand RNA (~30 kb) as its genome. It encodes at least 10 structural (S, M, E, and N) and nonstructural proteins. The spike (S) protein protrudes from the virion surface and binds to human angiotensin converting enzyme 2 (ACE2) via the receptor binding domain (RBD) of the S protein to allow the virus to gain cell entry (*3*). Given this function, the S protein is a promising target for the development of a vaccine and monoclonal antibody therapy. The 3D structure of the S protein and its binding mode to human ACE2 has been described (*4*), supporting vaccine development and analysis of monoclonal antibodies. The nucleoprotein (N) associates with the viral genome within the virus particle and is thought to be a good target for an immunochromatography-based rapid diagnosis kit. Both the matrix (M) and the envelope (E) protein are important for virus particle formation at the ER-Golgi intermediate compartment. However, the precise roles of these proteins are unknown because SARS-CoV-2 has just emerged. Therefore, we need to understand the virus and the disease through both clinical observations and laboratory experiments.

The alphacoronaviruses HCoV-NL63 and -229E and the betacoronaviruses HCoV-OC43 and -HKU1 are widely known as human pathogens for the common cold, whereas the betacoronaviruses severe acute respiratory syndrome (SARS)-CoV and Middle East respiratory syndrome (MERS)-CoV caused outbreaks with high case fatality rates (10% and 30%, respectively) in humans in 2002-2003 and 2014-2015, respectively (*5*). Large scale

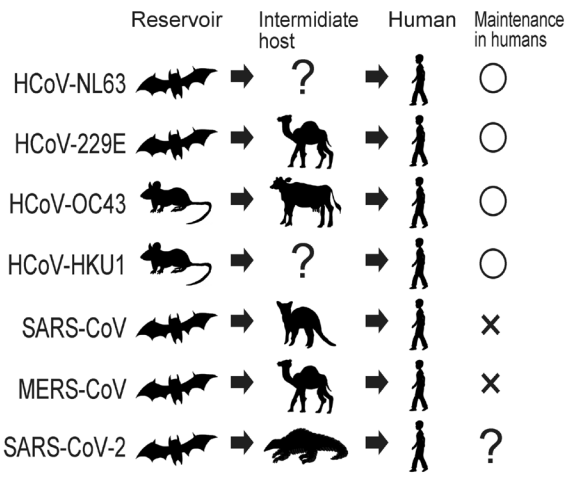

Figure 1. The putative reservoirs and intermediate hosts of seven CoV that have infected humans. The reservoirs and intermediate hosts of each Cov are listed. HCoV-NL63, -229E, -OC43, and -HKU1 cause human infections every year, whereas SARS- and MERS-CoV were not maintained in the human population until now.

virus surveillance in wild and domestic animals revealed that diverse CoVs related to HCoV-NL63, -229E, -OC43, and -HKU1 can be detected in bats and rodents, whereas CoVs closely related to HCoV-229E and -OC43 are found in camels and cattle (*5*), suggesting that bats and rodents are reservoirs of HCoVs, whereas camels and cattle are intermediate hosts (Figure 1). Similarly, bats are a reservoir of SARS- and MERS-CoV, whereas civets or camels play a direct role in transmission to humans as intermediate hosts for SARS- and MERS-CoV (Figure 1).

Although all six CoVs cause mild-to-severe disease in humans, the low virulent HCoV-NL63, -229E, -OC43, and -HKU1 viruses continue to transmit between humans, whereas the relatively highly virulent SARS- and MERS-CoV are not maintained in the human population; SARS-CoV has disappeared from the human population and MERS-CoV infection has been limited at the interface between humans and camels. We do not know what determines whether a novel coronavirus that emerges from animals will subsequently become established in the human population. Based on our scientific knowledge of other human coronaviruses, researchers immediately sought a reservoir and an intermediate host for SARS-CoV-2 and found coronaviruses genetically similar to SARS-CoV-2 in bats and pangolins (*3,6*). Genetic analysis showed that the viruses detected in pangolins are relatively similar to SARS-CoV-2 compared with those detected in bats, indicating that bats and pangolins likely serve as a reservoir and an intermediate host, respectively. However, further analysis is warranted to definitively ascertain which animals are reservoirs and intermediate hosts of these viruses.

It may be too early to discuss what will happen after the outbreak; we cannot predict whether SARS-CoV-2 will disappear from the human population like SARS-CoV or will continue to transmit between humans like the four common cold-related CoVs. Since the case fatality rate of COVID-19 is currently 2-3%, which is lower than those of SARS- and MERS-CoV but higher than those of the four common cold-related CoVs, we speculate that SARS-CoV-2 will be maintained in humans and remain the causative agent of COVID-19 after the outbreak. However, if we can contain SARS-CoV-2, it should disappear from the human population; although this possibility is rapidly diminishing due to the fact that the virus has already spread worldwide.

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Improved treatment capacity and quality of care: the effectiveness of the stroke prevention and treatment system in Shanghai, China from 2012-2017

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Abstract: Because of the high mortality, recurrence, and rate of disability of stroke, a stroke prevention and treatment system was instituted in Shanghai in 2012; this system includes 11 municipal hospitals, 25 district hospitals, and 240 community health centers. Community health centers focus on early screening in the community, health management of high-risk individuals, and secondary prevention and rehabilitation of stroke patients. Residents' health profiles are utilized by community health centers to proactively identify the population at higher risk. District hospitals are responsible for screening for vascular lesions in high-risk individuals, including carotid artery and intracranial artery screening, and standardized treatment of stroke patients. Municipal hospitals concentrate on complex and emergency care for acute onset stroke. The system specifies care for all stages of stroke management. The development of the system has improved the capacity of and quality of care for stroke patients. The rate at which patients undergo intravenous thrombolysis and the percentage of patients with a door-to-needle time of less than 60 minutes have increased significantly. However, the primary and secondary prevention of stroke is insufficient, the stroke rehabilitation system is incomplete, and the quality of care in primary healthcare facilities is limited. An evaluation system and payment mechanisms are needed to incentivize healthcare personnel to fulfill their responsibilities and to ensure the system's operation.

***Keywords*:** stroke, integrated care, treatment network

Introduction

Stroke, also known as a cerebrovascular accident or CVA, is an acute cerebrovascular disease. It happens when a blood vessel in the brain bursts or is blocked, thus obstructing the flow of blood into the brain and causing damage to brain tissues. There are two types of stroke: ischemic and hemorrhagic stroke. The former is also known as cerebral infarction, and it accounts for 85% percent of all stroke incidents. Stroke has become a global health problem due to its high incidence, mortality, recurrence, and rate of disability.

The 2017 Global Burden of Disease (GBD) Study indicated that cardiovascular diseases are the top killers around the world, and stroke became the leading cause of death and the leading cause of years of life lost in China in 2017 (*1*). The GBD Study suggested that stroke had a prevalence of 2,394/100,000 population, an incidence of 301/100,000 population, and a mortality of 149/100,000 population in China in 2017 (*1,2*). Data from China's Stroke Prevention and Treatment Report (2015) indicated that 15% of people over 40 are at high risk of stroke. In Europe and the US, the incidence of stroke is declining while it is increasingly worsening in China, with the incidence of stroke rising at an average rate of 8.7% every year (*3*). Moreover, the mean age at stroke is decreasing in China. Studies have indicated that the average age at stroke is 63 in China but 73.2 in the US (*4*).

In China, 2.7 million more people develop cerebrovascular diseases every year, and 1.3 million die of these diseases. A new stroke incident occurs every 12 seconds, and someone dies of stroke every 21 seconds (*5*). When a stroke occurs, it often develops too fast for patients to receive timely treatment, which means they are highly unlikely to recover completely, and the aftermath of the stroke will be with them for life. Stroke is also the leading cause of disability in Chinese adults. About 75% of stroke patients survive with varying degrees of disability, and 40% of those are severely disabled (*6*). Their quality of life is thus significantly diminished. Stroke patients suffer from physical and

motor impairments as well as from linguistic, cognitive, and emotional problems (*7*). Studies have indicated that risk factors for stroke include high blood pressure, diabetes, atrial fibrillation (*8*), hyperlipidemia, smoking (*9*), overconsumption of alcohol (*10*), aging, and hereditary factors (*11*). High blood pressure is the single most dangerous risk factor (*12,13*).

According to estimates, potentially modifiable risk factors are associated with almost 90% of the stroke burden (*14*) and effective interventions to control risk factors could reduce 75% of this burden (*15*). Hence, effective primary and secondary prevention is vital to reducing the incidence and mortality of stroke. Evidence-based medicine has proven that timely rehabilitation is the most effective way to prevent disability, and it is also an essential part of the organized management of stroke (*16*). Clinical research has indicated that early rehabilitation can help patients recover their limb function and it can reduce their likelihood of suffering complications like joint contracture, deformity, joint dislocation, or stroke-induced sarcopenia. It can thus enhance their quality of life. Therefore, assessing limb function in patients is essential to the treatment and management of their disease (*17,18*).

Intervening in stroke requires close collaboration among healthcare providers, preventive care, emergency care, clinical treatment, and rehabilitation services. The North Karelia Program enhanced primary prevention of and health education regarding stroke, resulting in 134 fewer expected fatal strokes or myocardial infarction in the first 5 years (*19*). Medical facilities in London were reorganized in 2010 to provide centralized acute stroke care. Patients with acute onset stroke are sent to a stroke unit for assessment and treatment and once stable are transferred to a nursing home or sent home for community rehabilitation. After the system was instituted, the risk-adjusted mortality 3, 30, and 90 days after admission decreased significantly (*20*). However, these services are physically separated in Shanghai's healthcare system. For instance, risk factor management and primary prevention are provided by community health centers under the technical guidance of centers for disease control, while clinical treatment and emergency care are provided by hospitals, and specialized nursing hospitals are responsible for rehabilitation. These facilities are operated separately without collaboration and are unable to provide a continuum of care as the condition progresses.

Because of the high mortality, recurrence, and rate of disability of stroke, the preventability of predictors of stroke, and the fragmented care delivery system, a stroke prevention and treatment system (denoted here simply as the System) was instituted in Shanghai in 2012. The key issues that concerned people in the establishment of the System were: how to reduce the incidence of stroke, how to increase the rate of standardized treatment within 60 minutes of stroke onset, and how to decrease recurrence, disability, and mortality. Here, the operation of the Systems and its effectiveness are described.

Stroke prevention and treatment system

Structure of the system

Shanghai's system of stroke prevention and treatment incorporates stroke prevention, intervention, and treatment. The System includes 11 stroke clinical treatment centers at the municipal level, 25 such centers at the district level, and 240 community health centers (Figure 1). These facilities have been assigned different roles depending on their functions and capabilities:

First, a stroke prevention and treatment center has been established at Huashan Hospital, and an expert committee on stroke prevention and treatment has been created. As the core of technical support for the entire system, the center is responsible for devising standards and formulating policies related to stroke prevention and treatment and for technical training, quality control, and overall supervision.

Second, 11 municipal-level tertiary general hospitals in Shanghai have been selected to serve as stroke clinical treatment centers. These clinical centers provide standardized facilities and services, which include acute stroke monitoring equipment, a neuroimaging platform, an acute stroke treatment protocol, a consultation system, and a referral and consultation system for critically ill patients. In addition, a specialized support mechanism has also been created in accordance with administrative divisions to create a care network for medical interventions targeting high-risk populations and emergency transfer of stroke patients. These 11 clinical centers can accept high-risk individuals referred from district-level stroke treatment centers and perform therapeutic interventions, including intravenous thrombolysis within a certain time period, subarachnoid hemorrhage treatment, endovascular intervention, and carotid endarterectomy. At the same time, they are also responsible for providing technical support to district-level treatment centers, mainly in areas where secondary facilities are weak at, which include neurosurgery, vascular surgery, and neuro-intervention.

Third, a management network has been created to screen and intervene in high-risk populations. The network is centered around district-level hospitals and includes community health centers and district centers for disease control (CDCs). The intent is to create a medical network for stroke prevention and intervention within a particular administrative area. The network has the following components: *i*) Establishing district stroke clinical treatment centers around district central hospitals. These centers are responsible for screening for vascular lesions in high-risk individuals, including carotid artery and intracranial artery screening, for providing standardized treatment to stroke patients (early

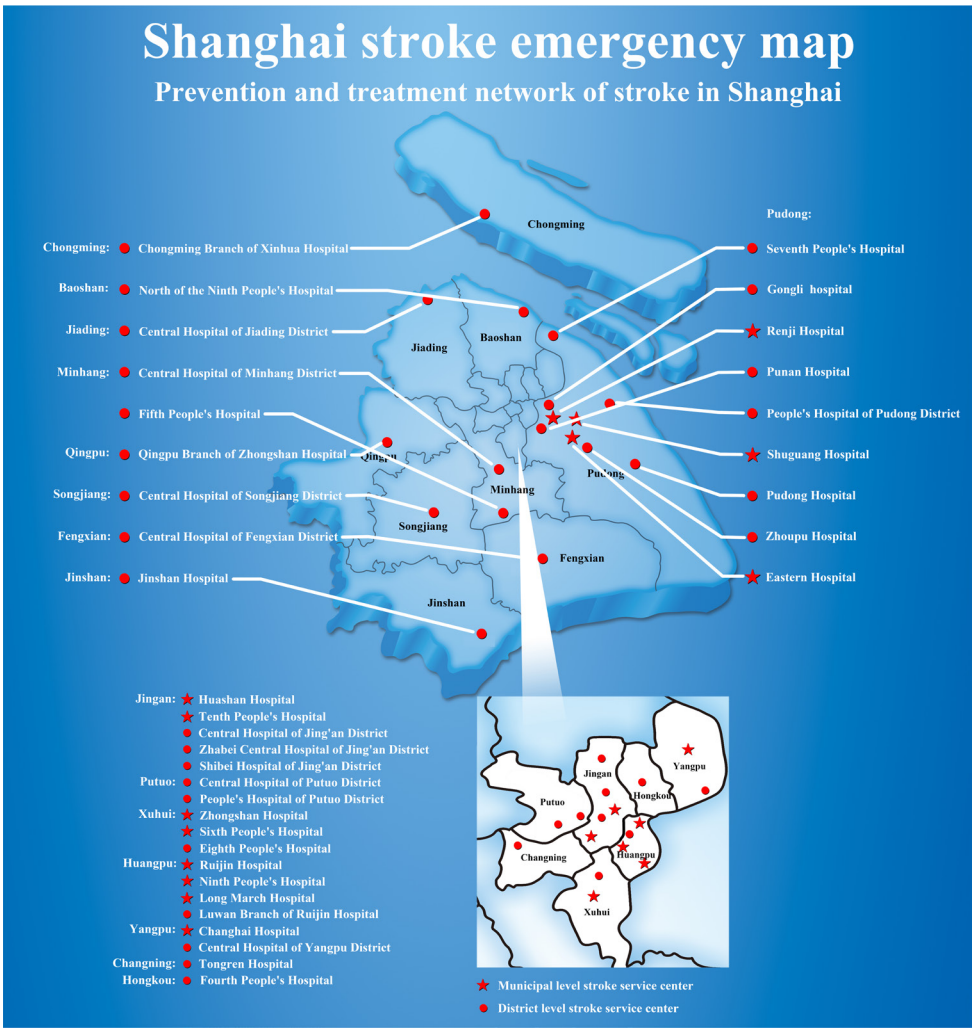

Figure 1. Hospitals participating in Shanghai's stroke prevention and treatment system.

thrombolysis or referral, cerebral hemorrhage surgery, and early rehabilitation), and for referring patients to corresponding municipal hospitals. *ii*) Providing stroke consulting services at community health centers. General practitioners and public health physicians are responsible for identifying high-risk individuals according to the "A-B-C-D" preliminary screening method (a validated predictor of stroke). The screening process also relies on residents' health profiles, outpatient consulting services, and health management (including chronic disease and family doctor services). Community health centers are also responsible for performing primary prevention including risk factor management, giving referral advice to high-risk individuals depending on screening results, and proactively using preventive care with traditional Chinese medicine (TCM) to provide stroke patients with means to recover from sequelae (Figure 2).

Cooperation with and support from professional facilities is also highly encouraged: the Shanghai CDC has officially incorporated stroke into its chronic disease management system, it has improved and standardized the screening process of high-risk populations, and it has established a reporting system for cerebrovascular diseases in Shanghai. Moreover, the Shanghai Medical Emergency Center has improved the system for transportation and allocation of patients with acute stroke, and it has also standardized its system for first-aid training. Furthermore, the Shanghai Health Education Institute, through its "12320" hotline, is promoting knowledge about stroke recognition and its management.

Standards and specified care

The System has set up specific care for all stages of stroke management (Figure 3), from first aid to emergency care, hospitalization (including early rehabilitation after acute stroke), outpatient and specialized follow-up care, and rehabilitation as well as community stroke consulting services and management of high-risk populations and stroke patients. This specified care includes: *i*) Standardized outpatient screening. Stroke risk assessment is used to identify high-risk individuals during regular service of related departments (including a physical exam); special attention is paid to patients with H-type hypertension and carotid artery disease. *ii*) Standardized care for

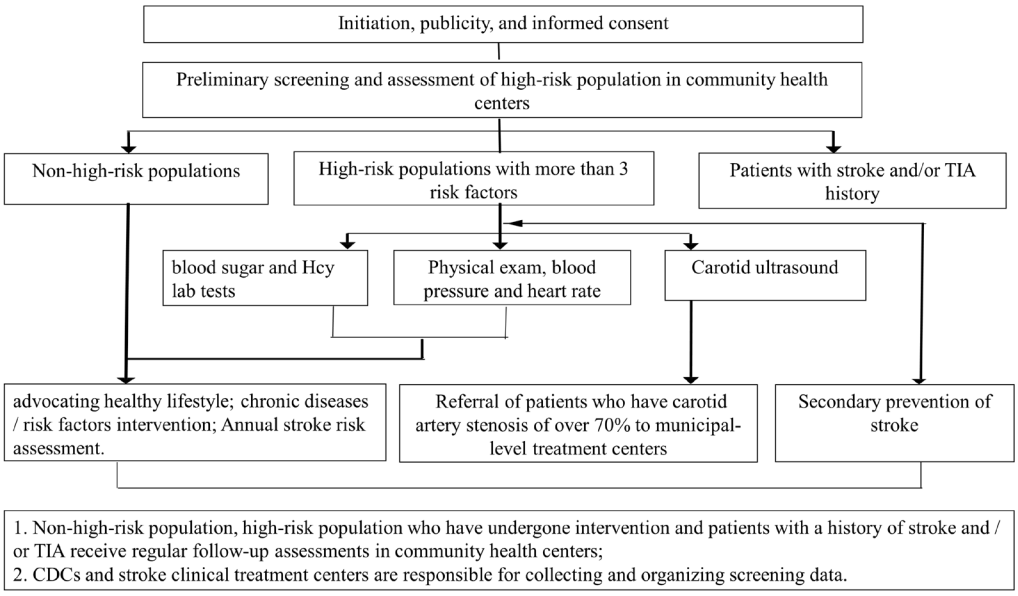

Figure 2. Process of screening and intervention in populations at high risk for stroke in Shanghai. TIA: transient ischemic attack; Hcy: homocysteine; Risk factors for stroke include: *i*) history of hypertension (≥ 140/90 mmHg) or is taking antihypertensive drugs; *ii*) atrial fibrillation or highly irregular pulse; *iii*) smoking; iv) abnormal blood lipids or unknown levels; *v*) diabetes; *vi*) lack of physical exercise; *vii*) overweight/obese (BMI ≥ 26 kg/m^2); *viii*) family history of stroke. A person with more than 3 risk factors is considered high-risk.

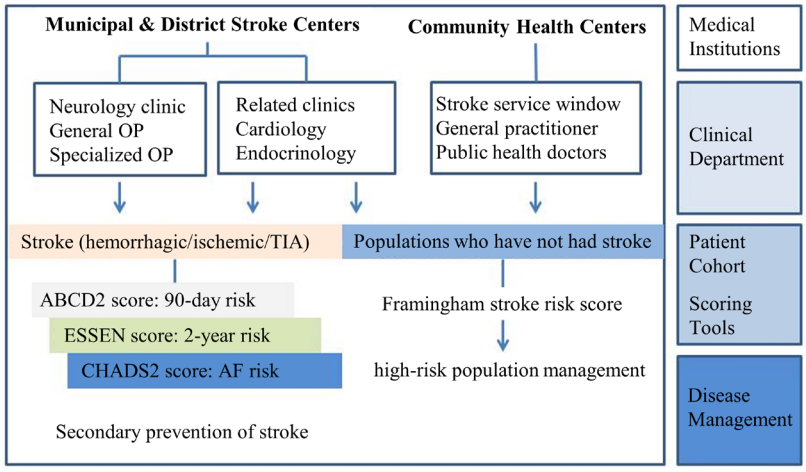

Figure 3. Specified care under the Shanghai Stroke Prevention and Treatment System. OP: outpatient; TIA: Transient Ischemic Attack; AF: atrial fibrillation; ABCD2 score: a validated risk stratification tool to identify patients at high risk of stroke following a TIA; ESSEN score: a risk score to predict recurrent cardiovascular events; CHADS2 score: predictors of the risk of stroke in patients with AF.

high-risk individuals in the community. Community health centers mainly perform primary prevention, using the "A-B-C-D" preliminary screening method to exam health profiles. "A" stands for an age over 55, "B" stands for blood pressure exceeding 140/90 mmHg, "C" stands for plasma homocysteine equal to or exceeding 10 μmol/L and low density lipoprotein cholesterol (LDL-C) over 3.2 mmol/L, and "D" stands for fasting blood sugar over 6.1 mmol/L. After identifying high-risk individuals, the Framingham Risk Score is rigorously used to assess and manage their health. The Framingham Risk Score includes age (over 55), systolic blood pressure, diabetes, smoking, atrial fibrillation (AF), and left ventricular hypertrophy (LVH). These parameters are used to identify individuals with a ten-year risk between 6% and 10%. These individuals will then receive primary prevention, namely a daily dose of aspirin 100 mg. *iii*) Standardized first aid. A 60-minute circle of life has been built based on Shanghai Emergency Center's transit route – the "green channels" – in order to raise the rate of standardized treatment. First aid is required to increase its 60 minutes' reach

Table 1. Quality of and capacity for stroke treatment in Shanghai from 2015 to 2017

Indicators	2015	2016	2017
Number of patients suffering an ischemic stroke	5,240	7,506	6,955
Number of patients undergoing intravenous thrombolysis in 11 municipal stroke centers	639	944	1,045
Number of patients undergoing intravenous thrombolysis in Shanghai	≥ 1,400	≥ 1,800	2,443
Median DNT in 11 municipal stroke centers (minutes)	65	65	59
Median DNT in Shanghai (minutes)	87	70	65
Percentage of patients with a DNT of less than 60 minutes (%)	41	40	50

to stroke treatment centers at all levels; physicians with emergency medical training are also needed to identify acute stroke. Treatment centers in the area need to be informed so that they can make preparations, and the vital signs of patients need to be continuously monitored. *iv*) Standardized emergency care for stroke. Stroke treatment centers at all levels should be able to finish overall assessment and make a treatment decision concerning an acute onset stroke within 60 minutes. All stages of clinical treatment should be more effective: lab tests should take no more than 45 minutes and a head CT scan should take less than 30 minutes so that patients who have had a cerebral infarct up to 4.5 hours earlier can receive a timely assessment and the chance to undergo intravenous thrombolysis and/or timely referral to Neurosurgery or to another hospital. Emergency physicians need to be trained to diagnose and treat acute stroke. *v*) Standardized hospitalization. In order to reduce complications and facilitate early prevention of stroke recurrence, key indicators of quality are used in accordance with the Chinese Guidelines for Secondary Prevention of Stroke and treatment guidance in the acute phase. The requirements of China's National Center for Stroke Care Quality Control and Management should also be followed. *vi*) Standardized outpatient care for stroke. Stroke outpatient centers should use inpatient assessment tools to provide care, including the ABCD2 score, Essen stroke risk score, and CHADS2 score for stroke risk in AF. Stroke risk scores are used to screen and manage individuals with no history of cardiovascular disease.

Separation of functions

First, municipal treatment centers provide district hospitals with technical assistance regarding neurosurgery, vascular surgery, and rehabilitation using TCM. They are also responsible for vascular neurosurgery, intracranial and extracranial surgery, and intravascular intervention.

Second, district treatment centers are equipped with stroke care teams of no less than 5 people, mainly consisting of neurologists but also including emergency physicians, neurosurgeons, and TCM practitioners. Specialized outpatient care for stroke is offered at least once a week, and 24-hour emergency green channels and vascular screening for high-risk individuals are provided. District centers also receive technical training from the Shanghai Stroke Prevention and Treatment Center and municipal treatment centers.

Last, community health centers provide stroke consulting services consisting of general practitioners, public health physicians, and TCM practitioners (no less than 5 people). Under the guidance of CDCs, community health centers manage high-risk individuals by means of community health profiles, standardized secondary prevention of stroke, and rehabilitation from stroke sequelae. Community health centers receive technical training from the Shanghai Stroke Prevention and Treatment Center and treatment centers at all levels.

Effectiveness of the system

Since 2011, the System has improved the capacity and quality of emergency care and care at the 36 stroke treatment centers in Shanghai. According to statistics from the Shanghai Stroke Prevention and Treatment System, the rate at which patients undergo intravenous thrombolysis and the percentage of patients with a door-to-needle (DNT) time of less than 60 minutes have increased from 41% in 2015 to 50% in 2017 (Table 1). The median DNT time at medical facilities of Shanghai dropped from 87 minutes to 65 minutes. The mortality of hospitalized stroke patients declined from 2.39% in 2016 to 2.1% in 2017.

Conclusion

Establishment of the System has enhanced the treatment capacity and homogeneity of care for stroke at different facilities, enabling patients to receive continuous services. However, primary and secondary prevention of stroke is lacking. High-risk population screening is not actively implemented in communities, and the overall quality of rehabilitation is still below par. An outcome-oriented evaluation system and a more sustainable funding mechanism should be devised to incentivize healthcare personnel to fulfill their responsibilities and to ensure the system's operation.

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Pathogenesis, clinical course, and recent issues in HIV-1-infected Japanese hemophiliacs: a three-decade follow-up

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Abstract: Nearly 30% of Japanese hemophiliacs were infected with HIV-1 in the early 1980s. They have unique characteristics compared to HIV-1-infected individuals through other routes, including date of infection of 1986 or earlier, mean age of nearly 50 years, and common co-infection with hepatitis C, but rarely with other sexually transmitted diseases. Antiretroviral therapy (ART) was introduced in Japan in 1997. The clinical courses before and after 1997 were quite different. Careful analysis of the pre-1997 clinical data allowed expansion of our knowledge about the natural course and pathogenesis of the disease. Switching to the second receptor agents proved critical in subsequent disease progression. HIV-1 continued to escape immune pressure, pushing disease progression faster. In contrast, ART was effective enough to overcome the natural course. Prognosis improved dramatically and cause of death changed from AIDS-related opportunistic infections and malignancies before 1997, to hepatitis C virus-related cirrhosis and hepatocellular carcinoma (HCC) around 2010, and again to non-AIDS defining malignancies recently. In most cases, hepatitis C was cured with direct acting antiviral therapy. However, HCV progressed to cirrhosis in some cases and risk of HCC is still high among these patients. Together with improvement in anticoagulants and aging of the patients, risk of myocardial infarction has increased recently. In addition, the numbers of patients with life-style related co-morbidities, such as diabetes mellitus, hypertension, and chronic kidney disease have been also increasing. Finally, stigma is still an important barrier to a better life in HIV-1-positive individuals.

***Keywords*:** natural course, escape from immune pressure, antiretroviral therapy, cause of death, HCV co-infection, co-morbidity

Introduction

The AIDS Clinical Center (ACC) was established at the National Center for Global Health and Medicine (NCGM) in April 1997 based on the out-of-court settlement among the HIV-1-infected Japanese hemophiliacs, the Japanese Government, and various pharmaceutical firms that supplied the HIV-1-contaminated blood products imported from the United States of America. Therefore, the main mission for the newly established ACC was to treat and care for the infected hemophiliacs. Subsequently, the role of the ACC was expanded to include the same treatment and care for all HIV-1-infected patients without any reference to the route of infection.

AIDS was first reported in homosexual men in 1981 (*1-5*). In the next year, AIDS cases were also identified in hemophiliacs (*6*). Seroprevalence was surveyed in Japanese hemophiliacs in the early 1980s, which showed that 29% of the tested hemophiliacs were positive for HIV-1 antibody (*7*). Based on the study, the time of HIV-1 infection of Japanese hemophiliacs was estimated to be 1983. Approval for the use of heat-treated blood products was granted in 1985, however, the use of contagious, untreated blood products was not prohibited before the end of 1986. Accordingly, Japanese hemophiliacs were thus exposed to HIV-1 and developed AIDS at that time. Based on the nationwide survey of coagulation disorders in HIV-1-infection, a total of 1,432 individuals had been infected with HIV-1 through contaminated blood products (*8*). At the time of writing this review, 33 years has passed since these patients were infected with HIV-1. Among them, 722 patients (50.4%) were still alive as of the end of 2017 (*8*). Their prognosis changed considerably in 1997 when the combination antiretroviral therapy (ART) was introduced in Japan. The causes of death were accordingly changed from AIDS-related before 1997 to other diseases thereafter.

We review here the natural course and pathogenesis of HIV-1 infection in Japanese hemophiliacs before 1997 and, then summarize the current and future clinical issues among these patients.

Differences between infected and uninfected hemophiliacs

As described, nearly 30% of Japanese hemophiliacs were infected with HIV-1 (*7*). In other words, the other 70% were not infected although they must have been exposed repeatedly to HIV-1 before 1987. HIV-1 can enter CD4+ T lymphocyte cells (CD4 cells) with second receptors of CCR5 and/or CXCR4 (*9*). Other studies confirmed that the delta-32 mutant of the CCR5 allele plays an important role in HIV-1 transmission and disease progression (*10*). Persons homozygous for delta-32 are well known to be resistant to HIV-1 infection. However, there are no Japanese homozygous for delta-32. In this regard, an international genome-wide study of resistance to HIV-1 infection in highly exposed uninfected hemophiliacs could not find any specific nucleoside polymorphisms related to the resistance (*11*). We do not have an accurate answer so far as to why some hemophiliacs were infected with HIV-1 but others were not at the host genetic level.

Viral tropism, pathogenesis, and disease progression

HIV-1 exists in the host as quasispecies, *i.e*., wide-range heterogeneity, in which the sequences of each virus have high similarities but are not identical to each other. HIV-1 quasispecies evolve over time throughout the clinical course. Isolation of HIV-1 with peripheral blood mononuclear cells revealed two distinct *in vitro* biological features of HIV-1; one isolate grows rapidly and yields high reverse transcriptase (RT) activity in the culture supernatant, and the other grows slowly with low RT activity (*12*). Accordingly, the rapid/high virus was termed the syncytium-inducing (SI) phenotype/T-cell line tropic virus and the slow/low virus was termed the non-syncytium-inducing (NSI) phenotype/macrophage-tropic virus. NSI variants are widely distributed in the body and are the predominant population throughout the asymptomatic to advanced stages. In contrast, the SI variants are often isolated from at least some patients with advanced disease (*13*) but are usually not isolated from chronic slow progressors. When isolated in the presence of high CD4 count, the CD4 count subsequently falls rapidly, with accelerated disease progression. Therefore, the emergence of the SI variants was thought to be a sign of disease deterioration. However, there was still a heated debate on whether the emergence of the SI variants was the cause or result of immunodeficiency. In a series of molecular studies from our laboratories, we found that a naturally occurring single amino acid substitution in the envelope variable 3 (V3) region alters the phenotype from NSI to SI (*14*) and that the basic amino acid arginine substitution at position 11 of the V3 region confers the SI phenotype (*15*). Using this molecular information, we investigated four hemophiliac patients (the clinical courses of two shifted to rapid progression while the other two remained slow progressors) with evolutional sequence analysis. The SI genotypes were only detected in the two rapid progressors just before CD4 depletion and thereafter, while the NSI genotypes were found in all patients throughout the clinical course. Interestingly, using phylogenetic analysis, we also demonstrated in another study that the SI genotypes were under stronger elimination pressure, compared with the NSI genotypes (*16*). Another study from our group confirmed the slow turnover of NSI virus at cell levels (*17*).

After identifying the second receptors (*9*), the SI genotype was found to be a CXCR-4 tropic virus (X4 virus) while the NSI genotype was a CCR-5 tropic virus (R5 virus) (*15*). In order to determine the pathogenesis of X4 and R5 viruses, we applied the deep sequencing method to investigate five slow progressor hemophiliacs who were ART-naïve for over 20 years (*18*). Among the five patients, two exhibited rapid decline in CD4 count during the clinical course and received ART, while the other three were untreated and remained ART naïve after completion of the study. Interestingly, in two patients with CD4 decline, X4 virus emerged before CD4 depletion. In the first case, the X4 virus was detected first in July 2006 when the CD4 count was 619/mm^3 and plasma RNA viral load (pVL) was 18,000/mL. The mean proportion of the X4 virus was only 0.9% at that time. However, in November 2007 (16 months later), the CD4 count decreased to 88/mm^3, pVL increased to 58,000/mL and the proportion of X4 virus increased to 17.4%. Then, ART was initiated in this patient. In the second case, the X4 virus was detected first (90.5%) in January 2009 when the CD4 count was 221/mm^3 and pVL was 530/mL. In November 2011, the CD4 count decreased to 44/mm^3, pVL increased to 11,000/mL, and the proportion of X4 virus decreased to 16%. Then, ART was initiated. Although the V3 sequences of X4 virus in both cases were quite unique and similar to each other, phylogenetic tree analysis showed that each X4 virus evolved from R5 virus in each patient independently. Our results suggest that the emergence of X4 virus preceded disease progression.

Immune pressure, escape mutations and disease progression

The natural course of HIV-1 infection has been well described in large cohorts before the ART era (*19*). There is a consensus that the asymptomatic period usually lasts around 10 years before development of AIDS. However, disease progression varies widely among patients and depends on a variety of factors, such as the viral factors described above. Another important factor is human leukocyte antigen (HLA). There is strong evidence that HLA-B*57/5801 and HLA-B*27 are associated with slow disease progression (*20*). However, both alleles are very rare in Japanese. After extensive studies on the association between HLA and disease progression

in Japanese hemophiliacs, Takiguchi and coworkers (*21*) concluded that HLA-B*5101 was a protective allele. They showed that HIV-1-specific cytotoxic T cells (CTL) restricted by HLA-B*5101 can strongly suppress HIV-1 replication *in vitro* and concluded that the presence of HLA-B*5101 allele in hemophiliac patients was significantly associated with slow disease progression. However, they also found that HLA-B*5101-restricted immunodominant CTL epitope Pol283 selected mutations at position 8 (position 135 of reverse transcriptase). Four amino acid mutations at position 135 (I135R, I135T, I135L, and I135V) were identified. Among them, the virus with I135V had a high fitness cost, but the others had the same replication capacity compared with the wild type virus. A proportion of slow progressors among the Japanese hemophiliacs had I135V virus and their pVL was extremely low probably due to the slow replicative capacity of the virus (*21,22*). In contrast, when the I135X (T, R, or L) emerged in patients carrying HLA-B*5101 and was then transmitted to their partners who did not carry HLA-B*5101, it existed in the new host in the absence of HLA-B*5101 selective pressure. This means accumulation of the mutant I135X in the peripheral circulation of HIV-1 in Japan (*22*). Actually, a significantly rapid disease progression was recorded in 59 acutely infected patients (infected with HIV-1 after 1997), relative to that of hemophiliacs (infected with HIV-1 before 1986) (*23*), probably due to lack of protection offered by HLA-B*5101 against disease progression in the recently infected Japanese (*22*). In this regard, adaptation of HIV-1 to escape HLA class I has deteriorated over time worldwide (*22*). If the highly active ART had not been established, the natural course of the disease could have probably accelerated, and some patients would have developed AIDS faster than before (*23*).

Anti-retroviral therapy before and after 1997

HIV-1 was first isolated by Barré-Sinoussi in 1983 (*24*) and the strong *in vitro* inhibitory effect of the first anti-retroviral agent, azidothymidine (AZT), against HIV-1 was demonstrated by Mitsuya in 1985 (*25*). A double-blind, placebo-controlled trial of 1,500 mg/day of AZT was conducted in the United States and the successful results of the 8-24 week treatment was published in July 1987 (*26*). In Japan, AZT was immediately approved without any local clinical trials and was made available for clinical use under the Japanese National Health Insurance at the end of 1987. However, the use of AZT at the recommended dose of 1,500 mg/day by our Japanese hemophiliacs was associated with severe bone marrow suppression. Similarly, the same severe side effect was also reported in some patients in a clinical trial in the United States (*27*). Prior to the availability of AZT in Japan, we conducted a small pilot trial of interferon-α (IFN-α) in 1987 in the early stage HIV-1 infection. The trial involved treatment of HIV-1-infected hemophiliacs with IFN-α at 3×10^6 units, three times a week. While side effects of IFN-α were limited, the treatment was unsuccessful (*28,29*). In contrast, daily treatment with IFN-α using a much higher dose of 35×10^6 units of successfully controlled plasma HIV-1 P24 (P24) antigen, in a randomized placebo-controlled trial in the United States (*30*), although severe side effects, such as high fever and general fatigue, were noted. At that time, no method was available to measure pVL and P24 antigen was the only virologic surrogate marker used to monitor clinical efficacy. Considered together, the high dose IFN-α was effective but toxic, whereas the low dose was ineffective.

Subsequent research led to a complete shift in treatment from the use of IFN-α to anti-retroviral agents. As described, although 1,500 mg /day of AZT was effective, it was associated with substantial toxicity (*27*). Therefore, a lower dose of AZT (600 mg/day) was tried in a randomized clinical study in the United States and the results showed the same efficacy but with less toxicity (*31*). In Japan, we also conducted a randomized clinical trial using a lower dose (400 *vs*. 800 mg/day) of AZT and the results of 400 mg/day showed less toxicity and more beneficial effects (*32*). In that trial, 79% of the study participants were Japanese hemophiliacs. In late 1980s and early 1990s, several nucleoside reverse transcriptase inhibitors (NRTI) were developed (*33-36*) and used as monotherapies in the clinical management of hemophiliacs. The combination of two NRTIs was also used for some of them. However, the clinical efficacy of NRTIs was very limited unless they were administered for about one year. For this reason, most of the Japanese hemophiliacs underwent long-term mono- or dual-NRTIs and experienced treatment failures until 1997.

The 3-drug combination treatment [HIV-1 protease inhibitor (PI) plus two NRTIs], which was coined highly active ART (HAART), was introduced in clinical practice in 1996 in the United States (*37,38*). This treatment markedly improved the prognosis of HIV-1 infected patients (*39-42*). The clinical status of the Japanese hemophiliacs also improved significantly after the introduction of HAART in 1997 compared with that of before 1997 (*43*). Although the ACC elected to use PI-based ARTs for the treatment of HIV-1 patients during the 2000s, such treatment was associated with increased bleeding tendencies at unusual sites, such as intramuscular bleeding, in both hemophilia A and B (*44*). Luckily, the bleeding was controlled with the use of clotting factors. Subsequently, we started to add clotting factors to the PI-based ARTs for hemophiliacs. ACC adopted the use of integrase strand transfer inhibitors (INSTI) when they became available in 2008 for the treatment of HIV-1-infected hemophiliacs (*45*), in part due to the safety issue of HAART-related bleeding site effects.

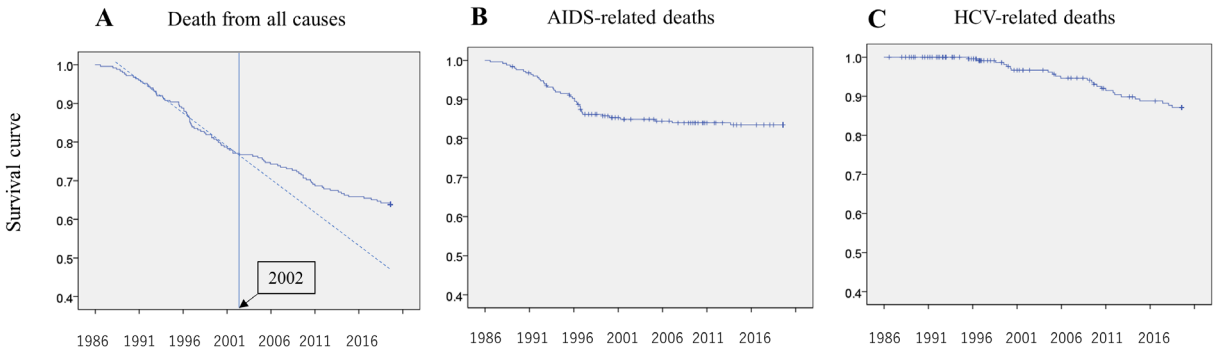

Figure 1. Kaplan-Meier survival curves. (A), Deaths from all causes (the dotted line is the approximate survival curve before 2002, the vertical line represents 2002); **(B)**, AIDS-related deaths; **(C)**, HCV-related deaths.

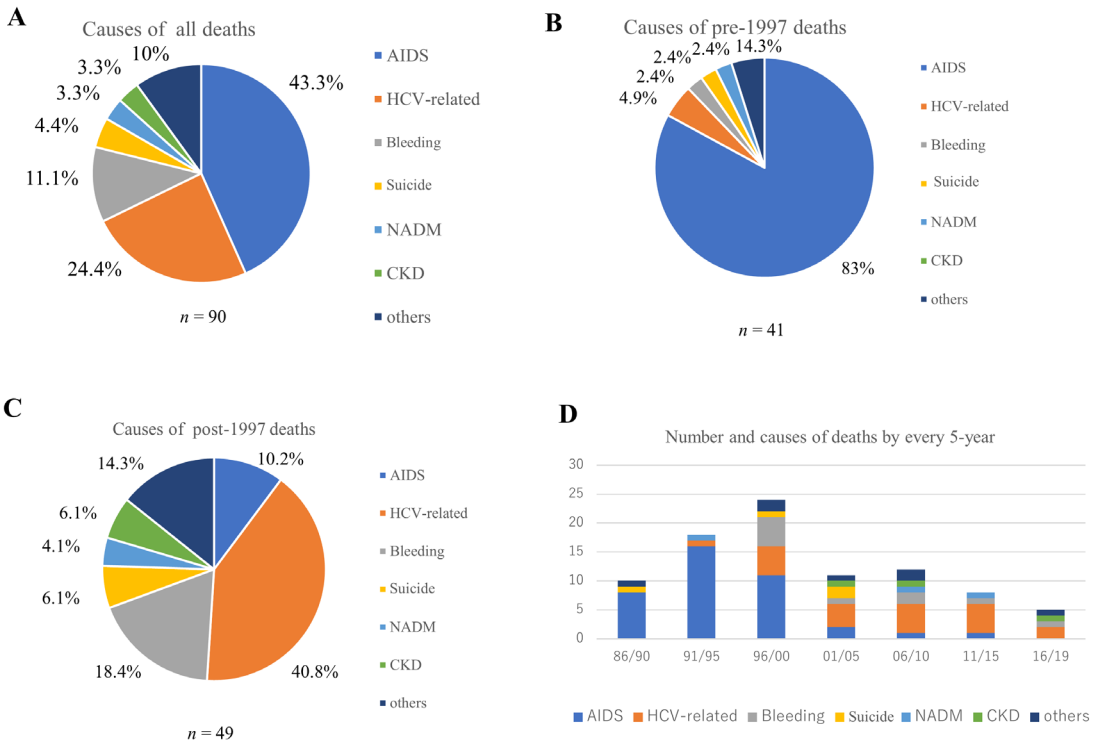

Figure 2. Causes of death in HIV-1-infected hemophiliacs. (A), Total number of deaths = 90; **(B)**, Causes of pre-1997 deaths (*n* = 41); **(C)**, Causes of post-1997 deaths (*n* = 49); **(D)**, Number and causes of death by every 5-year (Ordinate: number of deaths, abscissa: calendar years). Bleeding, intracranial bleeding; NADM, non-AIDS defining malignancies. CKD; chronic kidney diseases.

Mortality of HIV-1-infected hemophiliacs

A total of 249 patients (including 245 HIV-1-infected Japanese hemophiliacs and 4 patients with von Willebrand disease) were registered at the Department of Infectious Diseases, the Institute of Medical Science, University of Tokyo from April 1986 through March 1997 and at ACC, NCGM from April 1997 and thereafter. Among them, 90 patients died as of August 2019. Thus, the mortality rate in this group is 36.1%, which is far better than that of the entire population of Japanese hemophiliacs (*8*). The survival curves are shown in Figure 1. As described above, ART was introduced in Japan in early 1997. According to the Kaplan-Meier curve, the mortality rate improved after 2002 (Figure 1A), although the year 1997 saw a sharp fall in the number of AIDS-related deaths (Figure 1B). However, the number of HCV-related deaths has been gradually increasing since 2000 (Figure 1C).

Causes of death

Figure 2A provides details about the causes of death in our 90 patients. During the last three decades, the two major causes of death were AIDS (43.3%) and HCV (24.4%). However, the trend changed dramatically when the observation period was divided into before and after 1997. Before 1997 (Figure 2B), the majority of deaths (83%) were AIDS-related, whereas after 1997 (Figure 2C), these were largely (40.8%) HCV-related

deaths. The second cause of death was intracranial bleeding (18.4%), which might be related to the increased bleeding tendency associated with the use of PI-based regimens. To further dissect the causes of deaths, we analyzed the number and causes of deaths using 5-year bins (Figure 2D). The results showed bleeding-related deaths were the main cause between 1996 and 2000 when the first-generation PI-based regimen was mainly selected during that time period since no other options of effective HAART existed. Direct active antiviral (DAA) drugs against HCV were introduced in 2016 and almost all hemophiliacs with HCV infection achieved complete cure thereafter. The rate of HCV-related deaths gradually decreased after 2016, although some were still encountered probably because HCV hepatitis had already advanced to cirrhosis, at least in some patients. After 2001, although the number of deaths decreased (Figure 2D); suicide (6.1%) was the main cause in relation to poor mental health, followed by aging-related problems of non-AIDS defining malignancies (NADM) (4.1%) and chronic kidney diseases (CKD) (6.1%) (Figure 2C).

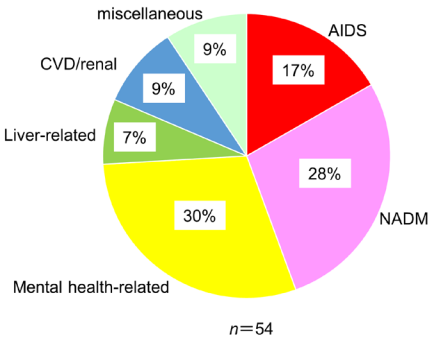

Figure 3. Causes of death in HIV-1-infected patients between 2016 through 2018. The data include all HIV-1-infected patients rather than hemophiliacs only. NADM, non-AIDS defining malignancies; CVD, cardiovascular diseases.

Impact of HCV co-infection

Almost all Japanese hemophiliacs were infected with HCV (*46*). It is well known that HIV-1/HCV co-infection accelerates HCV disease progression (*47*). Therefore, hepatitis C progressed to cirrhosis in some HIV-1-infected patients before they reached 40 years of age, which is otherwise unusual in the general population (*47*). Some of these HIV-1/HCV co-infected hemophiliacs who had already advanced to late-stage cirrhosis underwent living donor liver transplantation between 2001 and 2004 and the majority of the donors were their mothers less than 60 years old (*48*).

In the Japanese HCV-infected general population, HCV 1b is the main genotype followed by HCV 2a (*49*). However, in the Japanese hemophiliacs, the genotype pattern varied and corresponded to that described for the US, and included HCV 3, 4, and 6 (*personal information*). Therefore, for treatment of hepatitis C with genotype specific DAA, we faced difficulties related to the combination of these agents (*50*). However, we managed to achieve HCV cure in almost all HIV-1-infected hemophiliacs. As described above, the main cause of death was HCV-related after 1997 in the hemophiliacs. However, since almost all co-infected hemophiliacs had already been cured of HCV infection with DAA, HCV-related deaths have been decreasing in recent years (Figure 2D)

Non-AIDS defining malignancies (NADM) in the patients

Along with the ART-induced improvement prognosis of patients with HIV-1 infection and cure of HCV with DAA in the hemophiliacs, there has been a shift in the cause of death in recent years (Figure 2D). When we examined the causes of death in the past three years among our HIV-1-infected patients, one of the most important causes was non-AIDS defining malignancies (NADM) (Figure 3). The number of NADM has been increasing recently in our cohort (*51*). Although the precise reason for the steady increase in NADM is unknown, despite the decrease in other opportunistic infections, it can be speculated that recovery of immunosuppression is still incomplete (*52,53*). The importance of NADM was also confirmed in another large cohort study (*54*). Then, we conducted a double-cancer screening clinical trial between 2017 through 2019 in 69 Japanese hemophiliacs using ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET), chest CT, gastric endoscopy, occult stool blood and cancer biomarkers (*55*). In the first screening, we found 4 cases of malignancies (3 cases of thyroid cancer and one neuroendocrine tumor in the pancreas) with a mean age of 48.9 years. Thus, the prevalence of NADM was 5.8%. In the second screening, we identified two more new cases (one each of pancreatic cancer and hepatocellular carcinoma) within 1.2 years (68.2 person-years). Thus, the overall estimated incidence of NADM in the hemophiliacs was 2.99/100 person-years (*55*). Both the prevalence and incidence were unexpectedly high. In Japan, the number of hemophiliacs living with HIV-1 in 2017 is 718 (*8*). Therefore, we can predict that the annual number of new cases with undiagnosed cancer is 40 and that 20 new hemophiliacs per year will develop cancer. These results highlight the importance of cancer screening of HIV-1-infected hemophiliacs across Japan.

Neurocognitive impairment

The report of the CHARTER study of stable well controlled HIV-1 infected patients, concluded that HIV-1-associated neurocognitive disorders (HAND) have a

Table 1. Radiological findings and neurocognitive impairments in HIV-1-infected hemophiliacs

Items	Neurocognitive impairments +	-	*p*
MRI findings			
+	7	2	$p < 0.05$
-	20	29	
FDG-PET abnormalities			
+	15	22	n.s.
-	12	9	

A total of 58 HIV-1-infected hemophiliacs underwent the neuropsychological (NP) test battery, brain MRI, and FDG-PET. The correlations between radiological findings and neurocognitive impairments were examined by the χ^2 analysis. Neurocognitive impairments were examined by the 14 NP tests that assessed 8 cognitive domains used in the J-HAND study (*58*). Positive MRI findings: scars of faint intracranial bleeding. MRI, magnetic resonance imaging; FDG-PET, 18F-fluorodeoxyglucose-positron emission tomography; n.s., not significant.

strong and negative impact on prognosis (*56*). The same study found high prevalence of HAND (47%) among patients confirmed with a neuropsychological (NP) test battery. We also conducted a nationwide surveillance of HAND in Japanese patients (J-HAND study) and found a prevalence of 25.3% (*57*). Detailed analysis of the results showed a specific decrease in neurocognitive domains during the aging process (*58*). However, the J-HAND study did not include any hemophiliac patients as they were excluded by the study design. In another study, we co-screened neurocognitive impairments including psychiatric dementia using FDG-PET (together with the study of cancer screening (*55*)), brain magnetic resonance imaging (MRI), and the NP test battery that was also used in the J-HAND study. Based on the J-HAND study criteria, the prevalence of neurocognitive impairment was 44.3%, which was nearly twice as high as that in HIV-1-infected patients (*57*). However, the impairment was significantly associated with MRI findings of scars of faint intracranial bleedings during their childhood (χ^2 analysis; $p < 0.05$) (Table 1). These results suggested that the prevalence was influenced by the combination of HAND and sequelae of intracranial bleeding. Interestingly, the FDG-PET findings did not correlate with neurocognitive impairment in this study.

Stigma and future clinical issues

More than three decades have passed since our Japanese hemophiliacs were infected with HIV-1. Their mean age is approaching 50 years. Life-style related or age-related co-morbidities, such as chronic kidney diseases, hypertension, and diabetes mellitus, are the main clinical issues, rather than HIV-1 infection, in our hemophiliac patients, similar to other patients infected with HIV-1 (*59*). Recent years have witnessed improvement in anti-coagulation therapy and lessening of bleedings in hemophiliac patients. In contrast, cardiovascular diseases such as myocardial infarction have also increased recently. Furthermore, these patients have suffered stigma and discrimination against not only HIV-1 but also hemophilia itself over a long period of time and this will continue in the future. Persistent psychiatric pressures over many years have induced mental health problems. We must pay attention to the relatively high suicide rate of 6.1% recorded after 1997 (Figure 2C). Comprehensive treatment including mental support is especially important and indispensable for HIV-1-infected hemophiliacs for better quality of life. More importantly, we have to achieve zero stigma in our society for people living with HIV-1.

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Supermicrosurgery for oncologic reconstructions

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Abstract: With advancement of microsurgical techniques, supermicrosurgery has been developed. Supermicrosurgery allows manipulation (dissection and anastomosis) of vessels and nerves with an external diameter of 0.5 mm or smaller. Because quality of life of cancer survivors is becoming a major issue, less invasive and functionally-better oncological reconstruction using supermicrosurgical techniques attracts attention. Conventional free flap reconstruction usually sacrifices major vessels and muscle functions, whereas supermicrosurgical free flaps can be transferred from anywhere using innominate vessels without sacrifice of major vessel/muscle. Since a 0.1-0.5 mm vessel can be anastomosed, patient-oriented least invasive reconstruction can be accomplished with supermicrosurgery. Another important technique is lymphatic anastomosis. Only with supermicrosurgery, lymph vessels can be securely anastomosed, because lymph vessel diameter is usually smaller than 0.5 mm. With clinical application of lymphatic supermicrosurgery, various least invasive lymphatic reconstruction has become possible. Lymphatic reconstruction plays an important role in prevention and treatment of lymphatic diseases following oncologic surgery such as lymphedema, lymphorrhea, and lymphocyst. With supermicrosurgery, various tissues such as skin/fat, fascia, bone, tendon, ligament, muscle, and nerves can be used in combination to reconstruct complicated defects; including 3-dimensional inset with multi-component tissue transfer.

***Keywords*:** microsurgery, supermicrosurgery, reconstruction, cancer, lymphedema

Introduction

Reconstructive microsurgery has developed to allow various tissue reconstructions after tissue defect or functional deficit after oncologic surgeries (*1-3*). Locoregional flaps are used to reconstruct soft tissue defects. Pectoralis major myocutaneous flap and deltopectoral flap were mostly used for head and neck reconstructions, and various local flaps were developed to reconstruct soft tissue defects in any body part (*2-4*). With development of microvascular anastomosis, free tissue transfer or free flap transfer became a choice for reconstruction (*5-7*). Myocutaneous flap, consisting of major vessel and muscle/fat/skin such as latissimus dorsi myocutaneous flap and rectus abdominis myocutaneous flap, played a major role in free flap extremity and breast reconstructions. Free flap transfer from a donor site distant from tumor ablation site allows simultaneous flap elevation during tumor resection, which results in shorter operation time. Unlike local flap, free flap is useful for a microsurgeon to inset a flap with more ease and safety. Although myocutaneous free flaps enable immediate reconstruction of wide defects, these flaps are associated with significant morbidities in donor sites because of sacrificing major vessels and muscle. Since myocutaneous flaps have large volume due to muscle, esthetic reconstruction with natural contouring is difficult in face/head/neck and extremity reconstructions (*4-8*).

With advancement of microsurgical techniques and anatomical knowledge, perforator flaps were developed, in which the muscle can be preserved (*8-12*). Rectus abdominis myocutaneous flap was replaced with deep inferior epigastric artery perforator (DIEP) flap for breast reconstruction. Anterolateral thigh (ALT) perforator flap has become a choice of flap for head and neck reconstructions. Various perforator flaps were developed, allowing less invasive and more esthetically pleasing reconstruction. However, optimal functional and esthetic reconstruction is yet to be established even with perforator flaps requiring major vessels for flap pedicle and recipient vessels; recipient site and donor site cannot be freely selected, because major vessels are required in both donor and recipient sites (*9-13*).

With further advancement of microsurgical techniques, more sophisticated micro-vessels' manipulation becomes possible. Half-millimeter vessels can be anastomosed, and the technique is named supermicrosurgery (*14-19*).

Supermicrosugery

Definition of supermicrosurgery has been changing

over time (*14,20-22*). It is considered feasible for supermicrosurgery to be defined as microsurgical techniques dealing with vessels with external diameter of 0.5 mm or smaller, because surgical techniques and clinical applications are significantly different.

Technically, microsurgery deals with 1-2 mm vessels and its techniques are basically the same as in conventional vascular surgery (*22,23*). Left hand's forceps are inserted into a vessel lumen to assist suturing by right hand; the left hand's forceps keeps the lumen open, and prevent back wall catching by the right hand's needle. On the other hand, in supermicrosurgery, even micro-forceps cannot be inserted into a supermicro-vessel, and a surgeon has to perform suturing only based on a needle tip's sensation without left hand assistance. Since supermicrosurgery requires very meticulous manipulations with the sensation of tip of a 50 micron needle, rigorous training is necessary to master this technique (*22-25*).

Clinically, various reconstructions can be performed only with supermicrosurgery. First, any small tissue can be re-vascularized (*14,26-28*). After oncologic resection of some body part, the distal tissue can be replanted with supermicrosurgical anastomosis if needed. Second, single fascicle of a nerve can be coaptated with supermicrosurgery (*14,19-29*). Supermicrosurgical neuroraphy allows less invasive and fascicle-oriented nerve reconstruction. Third, any innominate vessels can be used as flap pedicle and recipient vessels (*14,18,20,30*). Supermicrosurgery does not require major vessels for donor or recipient sites, and allows free tissue transfer from anywhere to anywhere; true perforator flap transfer with perforator-to-perforator anastomosis (*17,30,31*). Three-dimensional multi-component tissues can be harvested and transferred with supermicrosurgery (*32,33*). Capillary-like supermicro-vessels nourishing various tissues can be dissected separately; chimeric flap transfer. Thickness-controlled flap transfer is possible with supermicrosurgical distal dissection of a perforator, allowing esthetic contouring reconstruction (*34*). Lastly, lymph vessels can be anastomosed (*14,17,35-45*). As collecting lymphatic vessels are usually smaller than 0.5 mm, supermicrosurgery is necessary for lymphatic reconstruction. Immediate or secondary lymphatic reconstruction becomes possible to prevent or treat lymphedema, lymphorrhea, and lymphocyst.

One of the most important clinical applications of supermicrosurgery in reconstructive surgery is versatile usage of superficial circumflex iliac artery (SCIA) perforator (SCIP) flap (*22,23,34,46,47*). SCIP flap is based on the superficial branch and/or the deep branch of the SCIA, and is a minimally invasive flap with a most esthetically pleasing donor site; and donor scar remains along the inguinal crease, which is concealable with underwear (Figure 1) (*22,23).* Since a SCIA branch is around 0.5 mm, supermicrosurgery is required for secure transfer of the SCIP flap. With supermicrosurgery, this optimal flap can be applied to various reconstructions in any body part as described below.

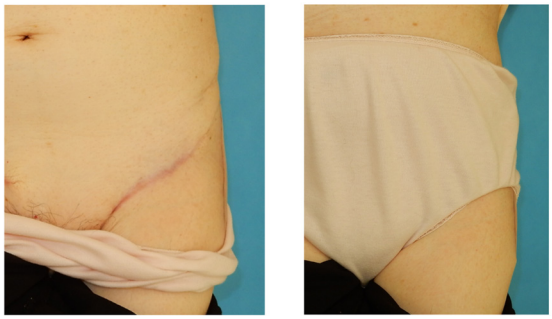

Figure 1. Superficial circumflex iliac artery perforator flap donor site scar is concealable.

Nerve and lymphatic reconstruction

In any body part, nerves and lymphatics exist, and may require reconstruction after cancer treatment. Reconstructive surgeons should consider these reconstructions for better functional outcomes.

Sensory and/or motor nerve injury can occur after oncologic ablation. Nerve defect should be reconstructed with vascularized nerve flap, because nerve flap has better postoperative nerve regeneration than non-vascularized nerve graft (*19,29,48*). Especially for motor nerve reconstruction, vascularized nerve flap is recommended due to 3 times faster regeneration (*48*). SCIP-based vascularized lateral femoral cutaneous nerve is a useful option for nerve flap transfer. Nerve flap can be used combined with skin flap for soft tissue defects. When a nerve defect is short, fascicular turnover flap based on the sacrificed nerve itself can be used for reconstruction, allowing autologous nerve reconstruction without donor site morbidity (*19,29*).

Lymphatic reconstruction is important for management or prevention of intractable lymphedema, lymphorrhea, and lymphocyst (*17,18,22,33,40*). Lymphedema is an obstructive lymphatic disease, and lymphorrhea/-cysts are a leakage disease. For lymphatic leakage diseases, precise localization of ruptured lymph vessels with near-infrared fluorescent lymphography and secure reconstruction is important (*17,40,41,49,50*). Since simple ligation may cause lymph flow obstruction and subsequent lymphedema development, lymphatic reconstruction should be performed for the leakage diseases as for obstructive disease (*17*).

There are mainly 2 lymphatic reconstructive methods; lymphatic anastomosis and lymphatic transfer. Lymphatic anastomosis includes lymphaticolymphatic anastomosis (LLA) and lymphaticovenular anastomosis (LVA) (*17,22,35-45*). In LLA, an affected lymph vessel is anastomosed to a nearby intact lymph vessel, whereas it is anastomosed to a vein in LVA (Figure 2). In both LLA and LVA, anastomosis should be done in an intima-

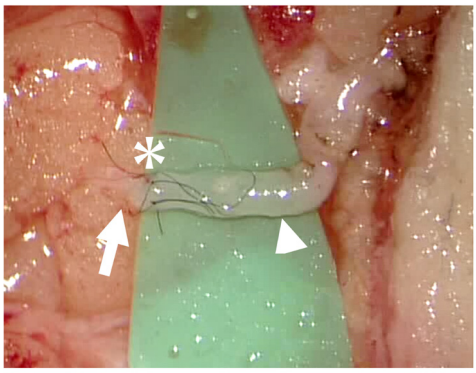

Figure 2. Lymphaticovenular anastomosis (asterisk) for the treatment of lymphedema, lymphorrhea, or lymphocyst. A lymph vessel (arrow) is supermicrosurgically anastomosed to a nearby venule or a vein (arrowhead) in an intima-to-intima coaptation manner. The vein looks like a lymph vessel, as lymph flows inside the vein making the vein translucent.

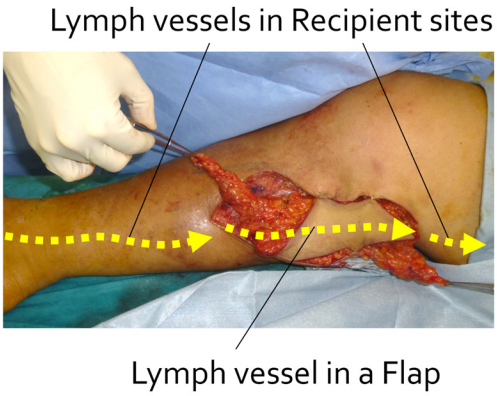

Figure 3. Lymph-interpositional-flap transfer (LIFT). LIFT bridges a gap of lymph vessel in a recipient site for lymphatic reconstruction.

to-intima coaptation manner with supermicrosurgery. Lymphatic transfer includes lymph node transfer (LNT), lymph vessel transfer (LVT), and lymph-interpositional-flap transfer (LIFT) (*14,18,22,49*). In LNT/LVT, vascularized lymph node/vessel is transferred to absorb lymph in a recipient site. In LIFT, a perforator flap including lymph vessels is transferred in a recipient site to bridge a gap between distal and proximal lymph vessel stumps caused by cancer ablation (*22,49*). SCIP flap can be used either as LNT, LVT, or LIFT (Figure 3).

Trunk reconstruction

Although conventional flaps are considered for reconstruction of a large defect requiring hard tissue reconstruction such as abdominal/chest wall or pelvic floor, true perforator flap or chimeric flap can be applied with less donor site morbidity (*1,22*). SCIP fascia flap can be used as a pedicled flap for abdominal wall and pelvic floor reconstruction, and as a free flap for chest wall reconstruction with or without a skin paddle (*22*). The deep branch of the SCIA should be used to transfer a vascularized fascia flap.

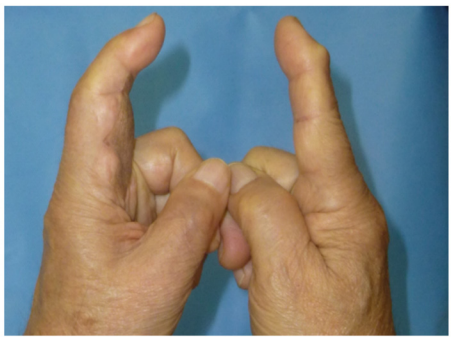

Figure 4. Super-thin flap for reconstruction of the finger.

Breast reconstruction

DIEP flap is the most popular flap for autologous tissue breast reconstruction, which is less invasive than rectus abdominis myocutaneous flap (*7-9*). Although preserving the rectus abdominis muscle DIEP flap requires dissection of the muscle and the intercostal nerves. The muscle dissection causes postoperative abdominal bulging and significant pain. Other perforator flaps allow even less invasive breast reconstruction without muscle dissection, which include superior/inferior gluteal artery true perforator flap, profunda femoris artery true perforator flap, lumbar artery true perforator flap, and SCIP flap (*22*). Flaps other than SCIP flap have relatively shorter pedicle unless proximal muscle dissection is not dissected. Both the deep branch and the superficial branch of the SCIA should be included to elevate an extended SCIP with enough bulk for breast reconstruction.

Hand and upper extremity reconstruction

Various pedicled perforator flaps can be used for relatively small defects, but requires additional skin grafting for donor site closure to reconstruct larger defects (*1,8,22*). True perforator free flap is functionally and esthetically better for reconstruction of medium to large soft tissue defects. SCIP flap plays an important role in upper extremity reconstruction, which allows thin and pliable skin reconstruction of the dorsum of the hand as super-thin or pure skin perforator flap, and simultaneous lymphatic reconstruction for prevention of lymphedema as LIFT (Figure 4) (*14,33,34,49*). Toe flaps are used to reconstruct digits, and domino free flap transfer is recommended for reconstruction of the toe donor sites (*16,26,31*). Chimeric SCIP flap with vascularized iliac bone is useful for complex reconstruction including the toe phalanx to preserve toe function and shape.

Lower extremity reconstruction

With supermicrosurgery, true perforator flap transfer with perforator-to-perforator anastomosis is a choice

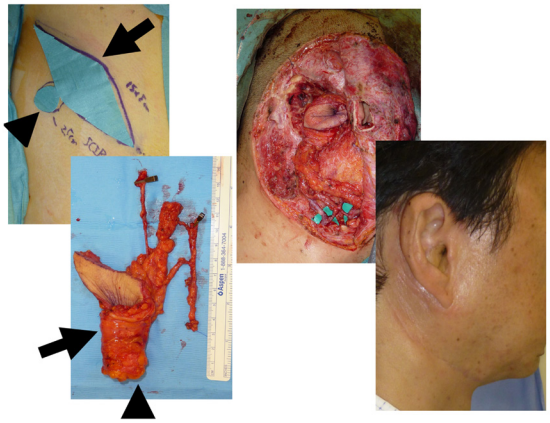

Figure 5. Superficial circumflex iliac artery perforator flap for reconstruction of the external auditory canal (arrow) and the tympanic membrane (arrowhead).

of reconstruction for distal lower leg and foot defects (*14,20,22*). Since any innominate vessels can be used as recipient vessels, supermicrosurgical true perforator flap transfer is applicable even for critical limb ischemia with no patent major vessels. For complex reconstruction of the ankle or the foot, chimeric SCIP flap is recommended, as it can transfer various tissues (the iliac bone, the deep fascia, the sartorius muscle, the inguinal lymph node, and the lateral femoral cutaneous nerve) without the need for a large recipient vessel (*22,33*). Since the lower extremities are likely to suffer from edema, lymphatic reconstruction plays an important role in improvement of postoperative quality of life. Therefore, LNT, LVT, or LIFT is recommended for reconstruction when a defect includes major lymphatic pathways as shown in Figure 3 (*22,33,49*).

Face, head, and neck reconstruction

Various true perforator flaps can be used for face, head, and neck reconstruction. For facial reconstruction, thoraco-acromial artery perforator flap is useful for color-matched re-surfacing (*14,22*). When a recipient vessel is not applicable in the ipsilateral side, contralateral vessels can be used as a recipient with a long pedicle flap. Deep branch-based SCIP flap can include a vascular pedicle as long as 22 cm, which is enough to reach the contralateral vessels (*22,46*). SCIP flap is useful also for reconstruction of the external auditory canal including the tympanic membrane (Figure 5). Facial nerve reconstruction has a significant impact on quality of life, and should be reconstructed simultaneously with soft tissue reconstruction as possible. Chimeric flap including vascularized nerve is a choice method for simultaneous facial nerve reconstruction rather than non-vascularized nerve grafting (*22,32,33,46*).

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International technical transfer of training systems and skills in emergency medicine and trauma management: experiences of the National Center for Global Health and Medicine, Japan

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Abstract: For over 20 years, the National Center for Global Health and Medicine (NCGM), Japan has been involved in international assistance for emergency medicine and trauma management in many countries, including Bolivia, Vietnam, Laos, Cambodia, and Mongolia. Among the NCGM activities conducted, the most important is technical assistance for the appropriate transfer of training systems and skills in life support management. In most of the target countries, the development and execution of customized simulation training suitable for each setting has successfully motivated trainees, who are healthcare workers responsible for improving emergency medical services in their home country. Moreover, the development of appropriate training systems for trainers selected from among capable participants has played a key role in the subsequent sustained conducting of training courses independent of NCGM involvement.

***Keywords*:** trauma system, injury surveillance, life support, essential trauma care, simulation, road traffic injuries

Introduction

A recent study reported that about 80% of the world's population reside in middle-income countries (*1*). When a country's income improves from lower-middle to upper-middle income, the incidence of road traffic injuries (RTIs) and other life-threatening events like heart attack and cerebrovascular accident or stroke dramatically increases because of the rapid development of motor transport and attendant changes in lifestyle. Notably, the number of deaths from RTIs reached 1.35 million in 2016 and has continued to steadily rise. If the present trend continues, RTIs are predicted to become the fifth leading cause of death by 2030 (*2,3*). Currently, more people die as a result of RTIs than from HIV/AIDS, tuberculosis, or diarrheal disease.

RTIs are at present the leading cause of death among children and young adults aged 5-29 years and the third leading cause of death of adults aged 30-44 years (*3*). Moreover, almost twice as many men as women die among those aged 15-44 years. This loss of the most economically productive population places an enormous economic burden on society. More than half of all RTI deaths involve vulnerable road users (*i.e.*, motorcyclists, pedestrians, and cyclists), and RTIs are predicted to be the third leading contributor to the global burden of disease and injury by 2030 (*4*).

In light of this, there is immense need for international assistance to these countries. Japan is well placed to offer such assistance because the country worked to overcome this same situation during the 1970s to 1990s (*5*) by improving pre- and in-hospital emergency medical services and developing training systems.

Bolivia

The National Center for Global Health and Medicine (NCGM), formerly known as the International Medical Center of Japan (IMCJ), began international medical cooperation in the field of emergency medicine in the city of Santa Cruz de la Sierra in 1994. The IMCJ worked to establish systematic integration of emergency medical services, "Sistema Integrado de Servicios Médicos de Emergencias (SISME)", which was run by the Japan International Cooperation Agency (JICA). SISME facilitated the development of inter-hospital liaisons, life support training programs for healthcare workers as well as citizens, and an ambulance transfer system activated by the 118 emergency call system. The NCGM also developed training for emergency physicians, creating a new medical specialty in Santa Cruz.

Vietnam

Rapid economic growth in Vietnam over the last decade or so has led to an increased incidence of RTIs. Between

2006 and 2010, RTIs resulted in 15,000 to 18,000 deaths each year in the country (*6*), with a high proportion of traffic collisions involving motorcyclists (58% in 2008-2009). Most of the deaths and injuries involved males (79%) ages 15 and 49 years. Overall, 42% of RTI deaths were caused by head injury (*6*).

The current authors conducted a cross-sectional study to ascertain the epidemiology of RTIs occurring in the City of Hanoi (*7*) using City of Hanoi police reports from 2006. Of 1,271 RTIs identified, about 40% involved people ages 20-29 years, and 63% of RTIs were motorcycle-associated incidents. Injuries occurred at two peak times: noon to 4 pm and 8 pm to midnight (Figure 1). "Hot spots" of RTIs and fatalities were identified in the city area and on main roads, with no easy access to major general hospitals for RTIs occurring along the two north-south main roads. Moreover, fatalities were significantly associated with the distance between the site of the injury and the hospital.

To help determine what assistance the NCGM could provide, the Guidelines for Essential Trauma Care (EsTC) (*8,9*) were used to evaluate trauma care and training levels at hospitals. These guidelines were published by the World Health Organization (WHO) and the International Association for Trauma Surgery and Intensive Care to improve organization and planning for trauma care at a low cost. Items in the guidelines cover trauma care and resources, with grading for each level of healthcare: "essential", "non-essential", "desirable" and "possibly required". For example, tracheal intubation is essential for tertiary and provincial hospitals but is desirable for district hospitals and basic health clinics. In northern Vietnam mainly, medical staff were interviewed, an EsTC-based checklist was distributed, and data were collected from 3 tertiary national hospitals, 6 provincial hospitals, and 11 district hospitals in Hòa Bình Province (*10*). Use of the checklist indicated that the level of trauma care was quite low in district hospitals. In Figure 2, the lower part of the bars indicates the number of essential items fulfilled and the upper part indicates the number of items that are non-essential, desirable, or possibly required. Orange, blue, and green bars indicate national, provincial, and district hospitals, respectively. Horizontal lines indicate the average number of essential items required for each level of health care. Results indicated that most of the hospitals at each level fulfilled

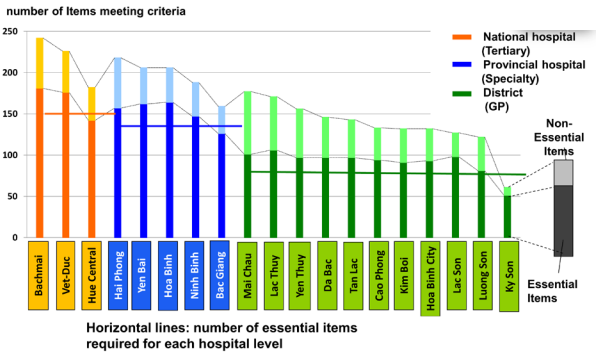

Figure 2. Survey of trauma care using the Guidelines for Essential Trauma Care (EsTC).

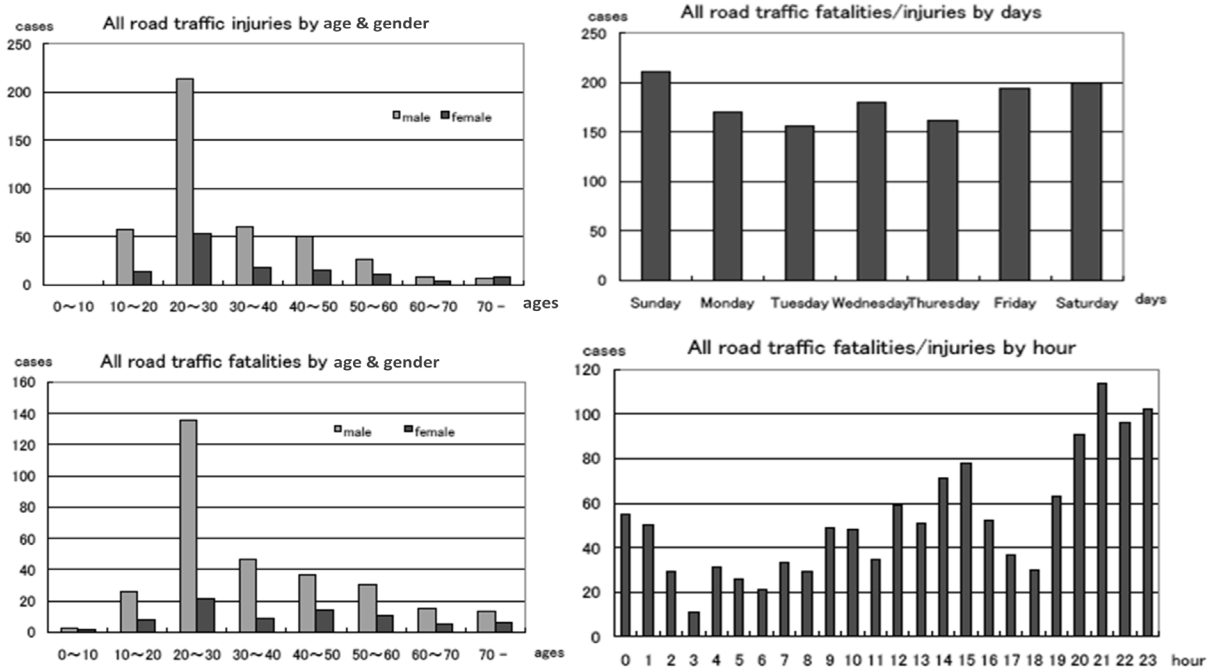

Figure 1. Distribution of road traffic injuries/fatalities in the City of Hanoi, Vietnam.

80% of essential items. The number of fulfilled criteria was found to correlate with total number of beds (r_s = 0.80, $p < 0.001$) and the total number of doctors (r_s = 0.711, $p < 0.001$). The distance to a hospital for referral was significantly correlated with equipment and supplies available ($r_s = 0.69$, $p = 0.016$).

The findings from use of the EsTC checklist were consistent with the state of trauma care at each hospital. At that time, there were very few emergency physicians in provincial and district hospitals. Newly trained emergency physicians in the country were working mainly at national hospitals in major cities like Hanoi, so many patients were concentrated at, or were referred to, tertiary hospitals in the major cities rather than at medical facilities far from the scene of the accident. A higher number of items were fulfilled by Bachmai Hospital (BH) and Hòa Binh General Hospital (HGH), where JICA hospital improvement projects had already begun. In contrast, Huế Central Hospital, fulfilled a very low number of items, even lower than some provincial hospitals, despite being a national hospital.

The NCGM began providing assistance by upgrading the ambulance transfer center and emergency department at each of the 3 general hospitals and by standardizing trauma care and introducing guidelines and simulation training. Basic life support training at the national hospitals was spread to the provincial hospitals. Advanced life support training was also conducted by inviting trainees from the 3 main hospitals to the NCGM hospital in Japan (*11*), which was very effective in increasing their motivation to improve life support management and emergency medical system in their home country. A comparison of pre- and post-training test results indicated that most trainees had 15% more correct answers after training, which was a statistically significant increase. A questionnaire completed by Vietnamese trainees revealed that they recognized the importance of confidence in cardiopulmonary resuscitation and other related skills as well as the importance of team dynamics. Almost all trainees became interested in simulation training and wanted to be trainers. Moreover, 94% of the Japanese trainers reported being interested in the international transfer of training skills, although they experienced communication difficulties mainly due to the language barrier. After this training, standard life support training was successfully spread to surrounding provincial hospitals based on the emergency department experience gained at BH. A challenge was also undertaken to adapt the trauma simulation training provided at HGH to a provincial model.

Laos

Laos is one of the lowest income countries in South-East Asia. As mentioned earlier, rapid economic growth and motorization usually result in high mortality from RTIs, and Laos is no exception. There is an urgent need for improvement in the country's trauma care. To obtain basic data on RTIs, a web-based injury surveillance system (ISS) was developed based on the WHO guidelines (*12*) for 3 national hospitals in Vientiane that have emergency departments. A cross-sectional study (*13*) found that in Vientiane, as in Hanoi, accidents involving young motorcyclists riding without helmets at night were a major issue (Figure 3). To obtain better registry data, training courses in trauma data management were conducted for Laotian healthcare workers, with assistance from the Khon Kaen Trauma Center that had collaborated with several earlier studies (*14,15*). Khon Kaen is a major city in northern Thailand located close to Vientiane. The training course seemed to be delivered much more effectively in Thai than by interpretation of the English spoken by Japanese trainers. This is because most people in Laos can understand Thai. Here again, courses in basic trauma care and disaster medicine were conducted. The workshop held with Laotian healthcare workers revealed many difficulties in ensuring sufficient funding to sustain training activities. In addition, the Internet infrastructure in Laos at that time was lacking, so the continuity needed for the ISS meant that web-based system had to be changed to standalone software that could be used offline. Using Thai in the training courses proved useful, but this option is not always available. Unfortunately, marked results have yet to be achieved in Laos thus far.

Cambodia

In 2015, road traffic accidents (RTAs) accounted for 2,265 deaths and more than 15,000 RTIs, 40% of which involved serious injuries. Since 2008, deaths resulting from RTAs in Cambodia have tended to increase and predominantly involve males (80%). The number of fatalities was estimated to have doubled between 2005 and 2015. The largest group of road users affected was motorcyclists (71%), followed by pedestrians (10%). Fewer than 50% of those involved in serious RTIs are transported by emergency ambulance (*16*). The need to enhance the emergency medical service (EMS) system in Cambodia has increased each year, and yet the training system for emergency medicine from prehospital care to in-hospital initial management has faults and has not met this need. In 2017, 3 emergency physicians from 3 national hospitals in Phnom Penh were invited to Japan for training as medical directors. Just after their return, they entered a training of trainers (TOT) program as part of a standard pre-hospital EMS training program for 27 trainer candidates in Phnom Penh. A "medical rally" was conducted as a form of integrated training. Four outstanding candidates working at national hospitals were selected to participate. The following year, in 2018, those 4 doctors were invited to Japan to train as medical directors, and after their return home,

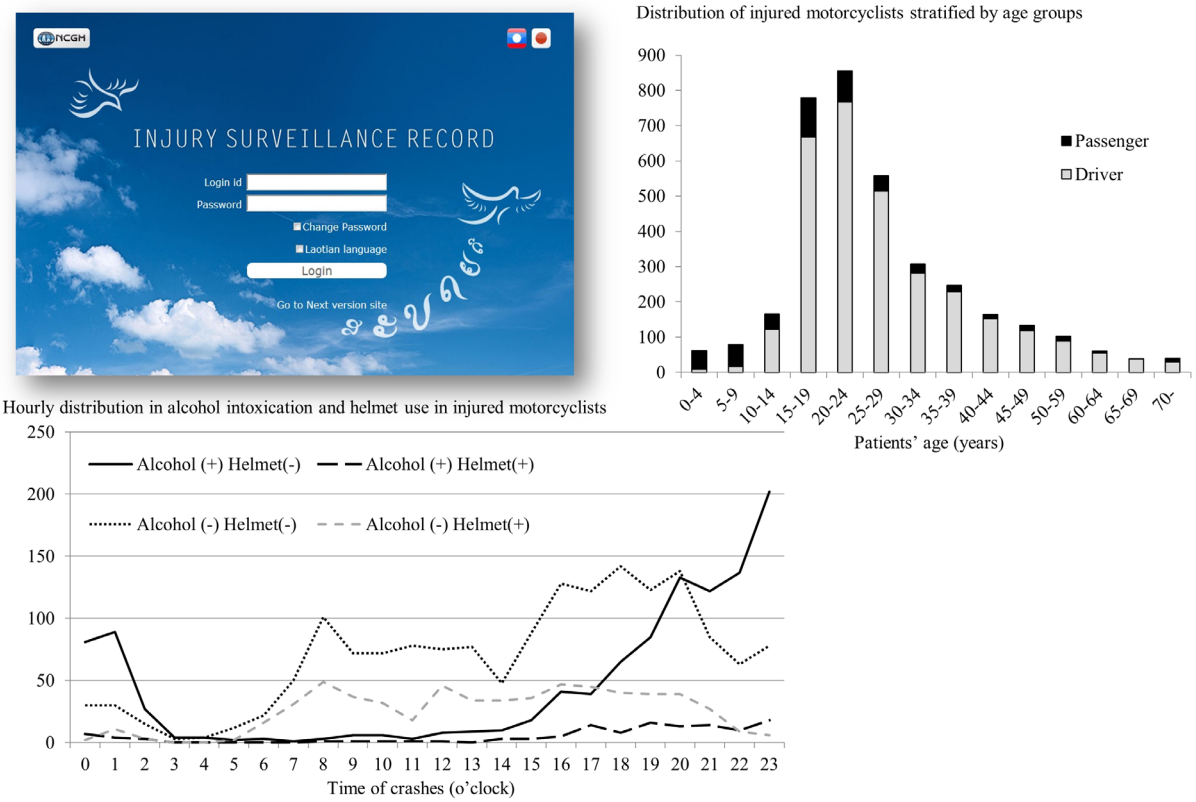

Figure 3. Introduction of the Injury surveillance system.

previously and newly trained trainers helped to conduct training in pre-hospital care and safe transfer for 25-32 healthcare workers from provincial and district hospitals in Siem Reap and Sihanoukville. In 2019, we expanded the EMS training was spread to Kampong Cham and Battambang provincial hospitals in collaboration with the now experienced Cambodian trainers. The effect of this training has been evaluated using the checklists of essential knowledge, skills, equipment, and supplies for prehospital providers (*17*), and the percentage of fulfilled items that are essential, desirable, and possibly required has increased.

Mongolia

The number of RTAs, crimes, and road safety violations is on the increase in Mongolia, and this trend is forecast to continue in the future (*18*). In 2015, JICA launched a project to enhance postgraduate training for health professionals in primary and secondary healthcare facilities. Its aim is to provide customized medical training that is feasible for doctors in Mongolia and sustainable. A major focus of this project is the development of a training system for emergency medicine in primary and secondary healthcare facilities. In 2016, the Advanced Assessment and Life Support (AALS) course was introduced, with assistance from skill simulators and running costs provided by JICA. The course offers comprehensive simulation training with the ABCDE approach and resuscitation skills in life-threatening situations customized for Mongolian settings. Participants are enthusiastic about implementing, continuing, improving, and disseminating the AALS course on their own. The course is being successfully managed by Mongolian instructors in the Mongolian capital of Ulaanbaatar as well as at the provincial level, and this success has led to other educational programs being developed for nationwide life support training in Mongolia.

Conclusion

Conducting simulation training customized to individual countries seems to be a highly effective way to transfer knowledge and appropriate skills. TOT programs may play a key role in identifying capable participants and in achieving sustainable development. Whenever possible, training should not merely be conducted in the target country. Rather, training should be conducted in the assisting country and in each target country in turn in order to encourage a more proactive attitude among participants with regard to improving emergency medical services in their home country. Starting and continuing training independently of the assisting country requires initial facilitation and some support in terms of equipment.

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Technical pearls in lymphatic supermicrosurgery

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Abstract: Lymphedema is becoming a major public issue with improvement of cancer survival rate, as the disease is incurable and progressive in nature, and the number of cancer survivor with lymphedema is increasing over time. Surgical treatment is recommended for progressive lymphedema, especially when conservative therapies are ineffective. Among various lymphedema surgeries, supermicrosurgical lymphaticovenular anastomosis (LVA) is becoming popular with its effectiveness and least invasiveness. There are many technical knacks and pitfalls in LVA surgery. In preoperative evaluation, indocyanine green lymphography is recommended for considering indication and incision sites. Intraoperatively, intravascular stenting method, temporary lymphatic expansion maneuver, field-rotating retraction, and several navigation methods are useful. The most important postoperative care is immediate compression after LVA surgery. Compression is critical to keep lymphatic pressure higher than venous pressure, allowing continuous lymph-to-venous bypass flow. These technical pearls should be shared with lymphedema surgeons for better lymphedema management.

***Keywords*:** lymphedema, microsurgery, supermicrosurgery, cancer, lymphaticovenular anastomosis, bypass

Introduction

Lymphedema is an edematous disease because of abnormal lymph circulation, and can be divided into primary and secondary lymphedema (*1-3*). Primary lymphedema is lymphedema other than secondary lymphedema, including genetic disorders such as Milroy disease (*1,4*). Secondary lymphedema develops after damage to the lymphatic system by such as trauma, lymph node dissection, infection, and radiation (*2-5*). With improvement of cancer survival rate, increasing number of cancer survivors suffer from lymphedema. Since lymphedema is incurable and progressive in nature, lymphedema management is becoming a major public health issue to be solved.

Treatment for lymphedema

Conservative treatments are mainstays of lymphedema treatment. The most important conservative therapy is compression treatment with either bandage or garments (*1-3*). Pressure gradient should be considered when bandage is applied. Therefore, appropriate bandaging requires specialized training. Garments specialized for lymphedema is recommended for most lymphedema cases. Other conservative therapies include skin care, appropriate exercise under compression therapy, and manual lymph drainage.

Even with rigorous conservative treatments, most lower extremity lymphedema cases and some upper extremity lymphedema cases cannot be controlled to stop progression of the disease. For such progressive intractable lymphedema refractory conservative treatments, surgical interventions are considered (*3,6-10*).

Lymphedema surgeries

Surgical treatments for lymphedema are largely classified into debulking surgery and physiologic reconstructive surgery (*3,8,11*). Debulking surgery aims to directly reduce volume by removing lymphedematous tissue; surgical resection and liposuction are applied (*3,11*). Physiologic reconstructive surgery includes lympholymphatic bypass, lymphovenous bypass, and lymphatic tissue transfer (*3,6-9,12-14*). Among various lymphedema surgeries, supermicrosurgical lymphaticovenular anastomosis (LVA) is the least invasive surgery effective for progressive lymphedema (*7,8-10*).

Lymphaticovenular anastomosis (LVA)

LVA surgery is a kind of lymphovenous bypass surgery, in which a lymph vessel is anastomosed to a nearby venule or a small vein (*6-10,11-15*). In other lymphovenous bypass surgeries, lymph vessel or node is inserted into or attached to a vein, and tissues other than the endothelium are exposed to venous blood, which

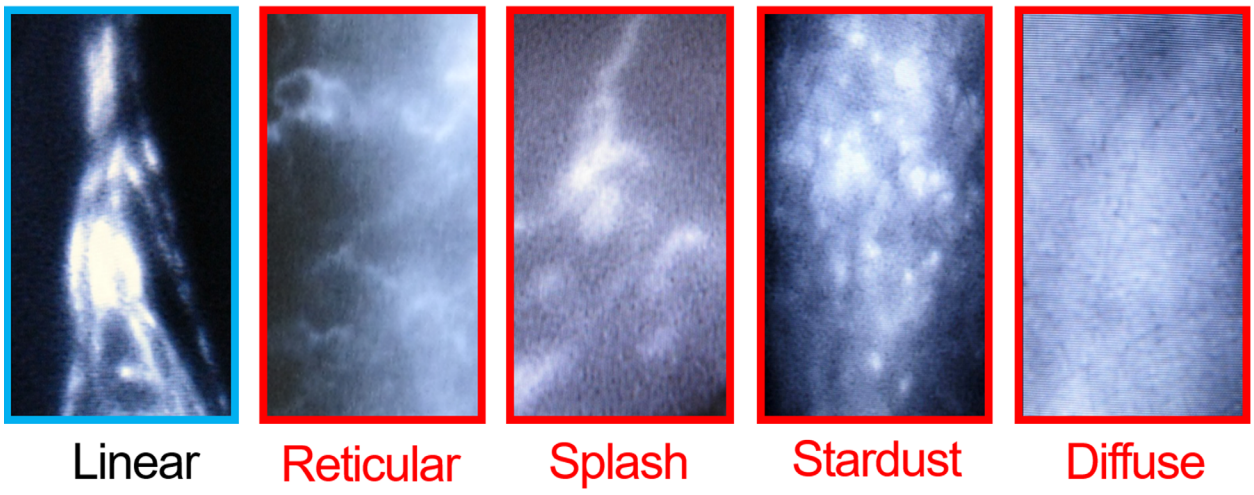

Figure 1. Indocyanine green lymphography findings. Normal lymphography findings is Linear pattern, whereas abnormal findings are Reticular, Splash, Stardust, and Diffuse patterns.

results in clot formation; thrombosis rate is high, and even deep vein thrombosis and pulmonary embolism have been reported (*3,6,15*). On the other hand, a lymph vessel is anastomosed to a vein in an intima-to-intima coaptation manner in supermicrosurgical LVA. The lumen of anastomosis site is covered by the endothelium, which prevents thrombosis even when venous blood contacts the anastomosis site. LVA can be performed under local infiltration anesthesia *via* a small skin incision, and no serious postoperative complication is reported (*6-9,12-14*).

Although LVA allows minimally invasive surgical treatments effective for compression-refractory progressive lymphedema, sophisticated microsurgical technique called supermicrosurgery is required (*6,7,8,16*). Supermicrosurgery is a microsurgical technique manipulating vessels with diameter of 0.5 mm or smaller, whereas conventional microsurgery deals with 1-2 mm vessels. A supermicrosurgeon has to master the feeling of sensation of a tip of a 50 micron needle for secure suturing (*6,7,17*). Understanding physiology, pathophysiology, and mechanism of lymphedema and LVA is also a key to successful management of lymphedema with appropriate perioperative management.

Preoperative management

Preoperative evaluation of lymph flow is critical for surgical indication and selection of incision sites. Indocyanine green (ICG) lymphography is the most useful imaging modality for lymph circulation. Near-infrared fluorescent images are obtained after intradermal or subcutaneous ICG injection at 2 phases; at an early transient phase observation immediately after ICG injection, and at a late plateau phase observation 2-72 hours after injection (*4,5,18,19*). This dual-phase ICG lymphography is called dynamic ICG lymphography (*18*). ICG lymphography findings are classified into Linear, Reticular, Splash, Stardust, and Diffuse patterns (Figure 1) (*5,18*). At a transient phase, Linear and/or Reticular patterns are seen. At a plateau phase, Linear, Splash, Stardust, and/or Diffuse patterns are seen. Linear pattern is a normal finding, representing collecting lymph vessels' flows. The other patterns represent abnormal findings called dermal backflow (DB). According to dynamic ICG lymphography findings, pathophysiological stage is determined, and recommended LVA sites are decided.

Table 1. ICG lymphography stage

Stage	ICG lymphography findings
Stage 0	Linear pattern only (No DB pattern)
Stage I	Linear pattern + Splash pattern*
Stage II	Linear pattern + Stardust pattern (1 region)**
Stage III	Linear pattern + Stardust pattern (2 regions)**
Stage IV	Linear pattern + Stardust pattern (3 regions)**
Stage V	Stardust and/or Diffuse pattern (No linear pattern)

* Splash pattern is usually seen around the axilla/groin. ** Upper/lower extremity is divided into 3 regions; the upper-arm/thigh, the forearm/lower-leg, and the hand/foot. DB: dermal backflow; ICG: indocyanine green.

Based on visibility of Linear pattern and extension of DB patterns, ICG lymphography stage is determined (Table 1) (*19,20*). ICG stage consists of stage 0 (no lymphedema), stage I (subclinical lymphedema), stage II (early lymphedema), and stage III/IV/V (progressed lymphedema). LVA is well recommended for lymphedema staged as ICG stage II/III/IV (*5-10,13,14,16*). LVA is considered as a prophylactic treatment for stage I subclinical lymphedema; LVA reduce progression risk of subclinical lymphedema from 40% to 0-5% (*2,3,7*). LVA can be performed for stage V lymphedema, but success rate to reduce lymphedematous volume is 30-50%. Vascularized lymph node transfer is recommended for stage V

lymphedema (*6,8,11*).

Regarding surgical sites for LVA, overlapping region is the most recommended. In overlapping regions, Linear pattern is seen at a transient phase, and DB pattern is seen at a plateau phase. Lymph vessels in overlapping regions are mild-moderately sclerotic affected ones which should be salvaged with LVA, and usually have high lymph flows (*5,10,16,20*). Using lymph vessels in overlapping regions, effective bypass effects can be expected. Lymph vessels in Linear pattern represents intact lymph flows, which should be preserved and not recommended for LVA. Lymph vessels in Diffuse pattern are usually severely sclerotic, not suitable for LVA (*8,16,20*).

Various techniques for lymphatic supermicrosurgery

Since lymph vessels used in LVA are approximately 0.3-0.5 mm in external diameter, supermicrosurgery is necessary to perform secure LVA. To address this technical challenge, various methods can be used. A nylon thread can be used as a stent to keep vessels' lumen open during anastomosis; intravascular stenting (IVaS) method (*6,7,16*). In IVaS method for LVA, several millimeter-long 3-0 to 7-0 nylon thread is inserted into a lymph vessel. IVaS method is available for all anastomotic configurations; end-to-end (EE), end-to-side (ES), side-to-end (SE), and side-to-side (SS) anastomoses (*6,12-14*). Various direction skin retractors are useful to fix a surgical field for better view; upward retraction is useful to do LVA in a medial aspect of extremity.

For SE or SS anastomosis, temporary lymphatic expansion (TLE) maneuver is helpful for easier lymphotomy (*12,13*). The most difficult procedure in SE or SS LVA is lymphotomy, a window creation on a side wall of a lymph vessel. TLE maneuver, proximal temporary lymph vessel cramping and distal limb massage, expands the lymph vessel, making it easier and safer to do lymphotomy. TLE is also useful to evaluate severity of lymphosclerosis. When a lymph vessel is expanded by TLE maneuver, it is less sclerotic and suitable for SE or SS anastomosis (*13,20*). Parachute technique, untied continuous suture, is helpful to perform secure SE and SS anastomosis.

Sometimes "one" lymph vessel show 2 or more lumens inside, confirmed after transection of the vessel. This is seen more frequently in cases with past history of lymphangitis, and represents attached multiple lymph vessels because of inflammatory adhesion. These lumens can be changed to one lumen by resenting the septum-like vessels' wall; mono-canalization technique (*6,13,16*). Mono-canalization allows easier anastomosis, with significantly less diameter discrepancy to a recipient vein; vein is larger in most cases.

Continuous lymph-to-venous flow is a key to long-term patency of LVA (*6,10,16,19*). There are 2 important points; prevention of venous reflux and increasing lymph flow into one recipient vein. To prevent venous reflux into an anastomosis site, valvuloplasty, neo-valvuloplasty, venous-branch-plasty, and valve-containing-venous grafting are useful (*12-14*). To increase lymph flow into a recipient vein, multiple-in-one (MIO) concept is important; multiple lymph flows are bypassed into one vein. In MIO concept, utilizing all anastomotic configuration plays an important role. Lambda-shaped anastomosis allows both proximal and distal lymph flows in one vein, in which proximal ES and distal EE anastomoses are performed (*6*). Sequential anastomosis diverts many lymph flows into one vein with the use of SS anastomoses. Other anastomotic combinations include, multiple SE anastomoses, all-star anastomoses, and ladder-shaped anastomoses.

Postoperative management

Immediate postoperative compression keeps anastomosis sites patent, with continuous lymph-to-venous flows *via* the anastomoses (*3,6,7,16*). In a lymphedematous limb, lymphatic system is closed; obstructed in the proximal region. When the limb is compressed, lymphatic pressure becomes higher. On the other hand, venous system is open, and venous pressure stays constant when compressed. Therefore, postoperative compression increases only lymphatic pressure, which leads to lymph-to-venous pressure gradient and continuous lymph flow *via* an anastomosis. In other microsurgical or supermicrosurgical vascular anastomosis, compression should be avoided. However, postoperative compression should be resumed immediately after operation in LVA surgery.

Conclusions

Supermicrosurgical LVA is minimally invasive surgical treatment effective for progressive lymphedema. ICG lymphography is useful to consider indication and skin incision sites of LVA. Various techniques help a surgeon to do this challenging supermicrosurgery. Immediate postoperative compression is recommended for better clinical results.

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Prevalence and incidence of HIV-1 infection in a community-based men who have sex with men (MSM) cohort in Ulaanbaatar, Mongolia

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Abstract: The number of HIV-1-infected men who have sex with men (MSM) Mongolian patients started to increase steeply just before 2011. We started collaborative work with community-based organizations that promote safer sex and HIV testing for MSM since mid-2010. Since early 2013, the Mongolian Government has implemented the treat-all strategy for MSM. To determine the efficacy of these countermeasures, we established an MSM cohort in the capital of Mongolia, Ulaanbaatar, in December 2013. HIV antibody was examined at every visit by rapid test. Syphilis was also examined to monitor their sexual behavior. Clients positive for either rapid test were referred to the National Center for Communicable Diseases, Ulaanbaatar, to confirm the results and treatment. Since safer sex promotion is one of the purposes of this cohort, HIV-positive clients were also eligible to participate. A total of 849 MSM were registered and 2,409 HIV/syphilis tests were conducted until December 2017. During this period, 499 (58.8%) clients visited the testing sites repeatedly. Among the 849 clients, HIV-1 infection was confirmed in 83 at registration (prevalence of HIV-1: 9.8%). One HIV-1 seroconverter was identified (from negative to positive), resulting in incidence of HIV-1 of 0.10/100 person-years (PY). Syphilis was positive in 144 cases at registration (syphilis prevalence: 17.0%), and 53 new syphilis infection cases were diagnosed during the same period, with an incidence of 5.66/100 PY. Despite the high prevalence of HIV-1, the incidence was very low. The results suggest that countermeasures for HIV-1 prevention seem effective in this cohort, however, we still need further strategies for syphilis control.

***Keywords*:** prevention, deep finger vein authentication system, syphilis

Introduction

Mongolia is a very low HIV-1 epidemic country (*1,2*). The Second-Generation HIV/STI Surveillance (SGS) was established in Mongolia in 2002 for a better understanding of the sexual behaviors that drive the epidemic and disease trends, and to use surveillance data to monitor and plan for the national response. Since the fourth round in 2005, the Ministry of Health and the National Center for Communicable Diseases (NCCD) conducted SGS. Based on the national surveillance data, the first HIV-1 case in Mongolia was reported in 1992 (*3*) and 250 HIV-1 cases had been reported by the end of 2017 (*4*). Apart from the first 5 cases, all other HIV-1 cases had been reported since 2005. Among the reported HIV-1 cases, 81% were males, 80% of them were men who had sex with men (MSM) (*3*).

In 2007, when our group in Tokyo started to collaborate with the NCCD, only a few HIV-1 infected cases had been reported. The reasons for the low epidemic could be the true status of the low epidemic or poor reporting due to lack of any surveillance system during that time period. In the same year, our research group conducted a community survey to evaluate the risk status of HIV-1 infection in Mongolia. A total of 1,415 blood samples from high-risk populations [*e.g.*, female sex workers (FSWs), MSM, mobile men, patients with active tuberculosis and male clients of the sexually transmitted infection (STI) clinic] and 1,050 samples from healthy adults were also collected and tested for HIV. Analysis of those samples showed no HIV-1 infection (*1*). The SGS survey conducted in the same year (2007) showed similar results of no HIV-1 infected cases except one positive in MSM. In the SGS

survey, 600 FSWs, 118 MSM, 750 mobile men and 1,902 male STI clients were tested for HIV (*5*). Therefore, we concluded that the prevalence of HIV-1 infection was really low in 2007. However, another survey in 2009 identified 3 new HIV-1 infection cases among 167 MSM (1.8%) (*6*) and that in 2011 found 21 new HIV-1 infection among 196 MSM (10.7%) (*7*). These results suggested that HIV-1 infection started to increase exponentially among MSM just before 2011, allowing us to conclude that intervention for HIV-1 prevention was urgently needed. Our molecular epidemiological analysis of HIV-1 infection using blood samples obtained between 2005 and 2009 identified the rapidly expanding HIV-1 transmission network among MSM, strongly confirming our conclusion (*8*).

To control the infection, we collaborated with local community-based organizations (CBOs) that provided safer sex education and promoted HIV testing for MSM since 2010. The Mongolian Government has implemented the treat-all strategy irrespective of CD4 count for MSM since early 2013. The objective of this study was to determine the efficacy of these countermeasures. For this purpose, we established an MSM cohort in Ulaanbaatar in order to document the incidence of HIV-1 infection among MSM after the countermeasures and monitored syphilis for reference of sexual activity.

Materials and Methods

Study design

MSM cohort was established in the capital of Mongolia, Ulaanbaatar, in December 2013 and followed at the end of 2017. Participants were recruited at two HIV testing sites in Ulaanbaatar. The eligibility criteria were MSM aged 20 years and older. A written informed consent was obtained from all participants. HIV-1-positive MSM were able to participate in this cohort because they could also undergo free syphilis testing, and enroll in safer sex education. Registration was conducted anonymously using a deep finger vein authentication system that connected with all study sites both in Mongolia and Japan and formed the network system. A study ID was automatically assigned based on the individual finger vein pattern and registered in the network system. All specimens and test results were treated with the study ID. Participants were able to receive free of charge rapid tests for HIV and syphilis at any time. The testing interval was left to the participants. Participants, who were positive for HIV and/or syphilis and needed confirmation tests, were referred to NCCD, Ulaanbaatar.

Recruitment of participants

Participants were recruited at Together Center and Rainbow Clinic in Ulaanbaatar. Together Center was operated by CBOs and provided voluntary HIV testing services especially targeting MSM. The medical staff of NCCD oversaw the HIV testing service and cooperated with CBOs activities.

Three major CBOs operating in Mongolia; Together Center, Youth for Health Center and Human Rights Youth Health Support, joined effort to form one community organization in 2015. Accordingly, Together Center moved to the center of Ulaanbaatar. At the same time, Rainbow Clinic opened as a free HIV testing site in NCCD adjacent to where Together Center had been located. Rainbow Clinic joined the cohort recruitment site from August 2015. Participants were able to use both sites.

Sample collection and HIV and syphilis testing

Blood samples were collected at Together Center and Rainbow Clinic. HIV and syphilis were diagnosed using the immunochromatography method (DAINA SCREEN HIV-1/2® and DAINA SCREEN TPAb® for the antibody for *Treponema Pallidum*, Alere Medical, Japan). The surplus blood samples after the rapid tests were kept at -4°C at each testing site and transferred to NCCD to confirm the results at the central laboratory of NCCD. The remaining serum samples were frozen at -80°C and periodically transferred to National Center for Global Health and Medicine (NCGM), Japan, to reconfirm the results by ARCHITECT® (Abbott Japan, Tokyo).

Data management

Central monitoring was performed every 3 months through the network system from Japan. The study monitors visited each site periodically (at least once annually throughout the study period) to ensure the study, records and reports adhered to the protocol and ethical guidelines. The monitors reported any protocol violations and other problems to the principal investigator and shared resolving these issues with collaborative researchers. The study progress and results were reported to the cooperative researchers, NCCD and CBOs every year.

Data analysis

Data of participants with HIV-1 or *Treponema Pallidum antibody* (TPAb) positive at study registration were included only in the HIV-1 and syphilis prevalence calculations. After excluding those participants who were HIV-1 antibody-positive at registration and those who received the test only once, the HIV-1 incidence was calculated using the number of seroconversions within the follow-up period. The total number of person-years (PY) represented the accumulated observation period from the registration date to the last test date. The incidence of syphilis was calculated by using the

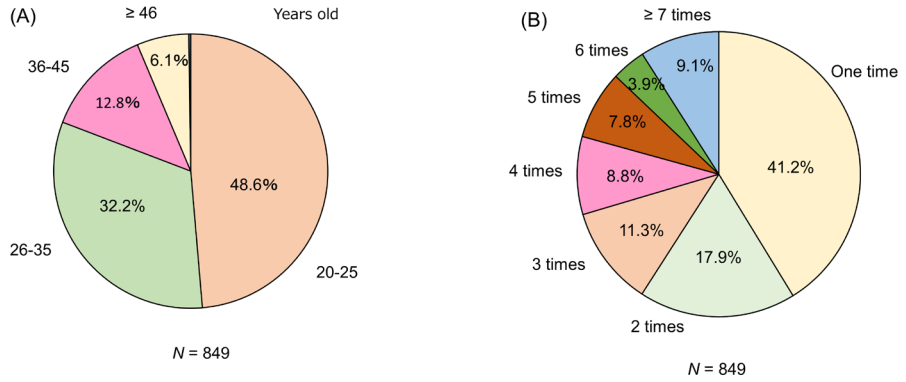

Figure 1. Background of study participants. (A) Age at registration. The study included 849 MSM with a median age of 26 years. **(B) Frequency of HIV and syphilis testing during this study.** A total of 2,409 HIV and syphilis tests were conducted in 849 participants. Nearly 60% of the participants received more than one HIV and syphilis test. In other words, 40% of the subjects were regarded as lost-to-follow during the study period.

Table 1. Prevalence of HIV-1 in our men who have sex with men (MSM) cohort each year

Year	Registered MSM (*n*)	Previously diagnosed HIV (*n*)	HIV diagnosis at registration (*n*)	Prevalence of HIV (%) (95% CI)
2014*	296	28	9	3.4# (1.0-5.7)
2015	187	7	2	1.1# (0.0-3.2)
2016	212	18	2	1.0# (0.0-3.0)
2017	154	15	2	1.4# (0.0-4.1)
Total	849	68	15	1.9 (0.9-2.9)

*2014 includes data of Dec 2013. #number of HIV diagnosis at registration/(number of registered MSM – number of previously diagnosed HIV) each year.

number of TPAb seroconversions in a manner similar to the calculation used to determine the incidence of HIV-1. The estimated prevalence and incidence were presented with 95% confidence intervals (95% CI).

Ethics statement

This study was reviewed and approved by the ethics committees of National Center for Global Health and Medicine (#NCGM-G-001426) on 20 June 2013 and the Ministry of Health, Mongolia (MOH-#3) on 25 October 2013. A written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. This study was registered with the University Hospital Medical Information Network Clinical Trial Registry (Registry number: UMIN000024089).

Results

MSM cohort in Ulaanbaatar

From December 2013 to December 2017, 849 MSM enrolled in this cohort and 2,409 HIV and syphilis tests were performed. At study registration, the majority of participants were relatively young with a median age of 26 years [Interquartile Range (IQR): 21-33] and 413 (48.6%) were 20-25 years old (Figure 1A). Of the 849 enrolled subjects, 499 (58.8%) underwent testing for HIV-1 and syphilis more than once during the study period (Figure 1B). The other 350 (41.2%) enrolled clients were tested only once and did not revisit the testing sites for re-testing, and were thus considered lost-to-follow. At each visit, the CBO staff provided HIV-1 prevention programs that included material on HIV-1 infection, promotion of repeated HIV-1 testing, and safer sex education to the clients. The detailed methods and contents of the HIV prevention strategies offered by the CBOs will be reported in the future.

HIV-1 prevalence and incidence

Table 1 shows the prevalence of HIV infection each year and Figure 2 illustrates the study flow and calculations of the prevalence and incidence of HIV-1. Among the 849 MSM participants, 68 had been diagnosed with HIV infection before participation in this study. In addition, 15 new HIV infection cases were confirmed at enrollment. Based on the total number of HIV infected MSM at enrollment of 83, the estimated prevalence of HIV-1 infection in this cohort was 9.8% (95% CI: 7.8-11.8%). The prevalence of each year was relatively low and stable over time (Table 1). Among the 766 cases who were HIV negative on the first test, 446 participants were tested more than once whereas 320 (41.8%) were

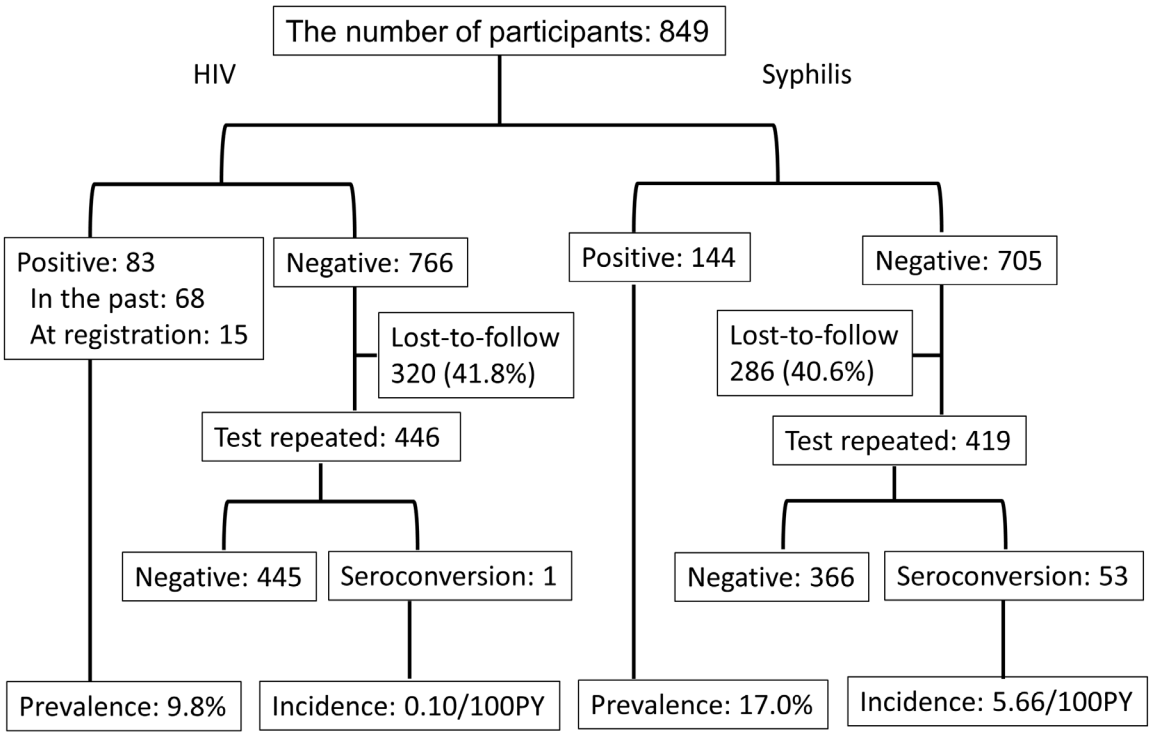

Figure 2. Prevalence and incidence of HIV and syphilis. HIV and syphilis tests were conducted in 849 participants. Among these, 83 participants were positive for HIV-1 (68 in the past and 15 at registration) and 766 were negative. Among the 766 participants, 446 were repeatedly tested for HIV and 320 (41.8%) were lost-to-follow. One seroconverted during the study. With regard to syphilis, 144 individuals were positive at registration and 705 were negative. Among 705 participants, 419 were repeatedly tested for syphilis and 286 (40.6%) were lost-to-follow, while 53 seroconverted during the study.

Table 2. Prevalence and incidence of syphilis in our men who have sex with men (MSM) cohort

Year	Registered MSM (*n*)	TPAb-positive at registration (*n*)	Prevalence at registration (*n*)	TPAb negative to positive (*n*)	Cumulative person year (PY)	Incidence (/100PY) (95% CI)
2014*	296	51	17.2	2	140.6	1.42 (0.39-5.19)
2015	187	30	16.0	9	224.9	4.00 (2.11-7.61)
2016	212	34	16.0	17	299.7	5.67 (3.54-9.08)
2017	154	29	18.8	25	271.9	9.20 (6.23-13.58)
Total	849	144	17.0	53	937.1	5.66 (4.32-7.40)

*2014 includes data of Dec 2013. TPAb: Antibody for *Treponema Pallidum*.

considered lost-to-follow for HIV testing. Only one HIV seroconversion was noted during the study period. The total observation period was 1,027.3 PY and the median observation period per patient was 2.28 (IQR: 1.30-3.53) years. The overall incidence of HIV was 0.10 (95% CI: 0.02-0.55)/100 PY.

Syphilis prevalence and incidence

Table 2 shows the prevalence and incidence of syphilis and Figure 2 illustrates the study flow and calculations of the prevalence and incidence of syphilis. At enrollment, 144 MSM were already TPAb-positive. Accordingly, estimated prevalence of syphilis (history of syphilis infection) was 17.0% (95% CI; 14.5-19.5%). Among the total of 705 syphilis-negative cases, as diagnosed on the first test, 419 were repeatedly tested whereas 286 (40.6%) were regarded as lost-to-follow for the syphilis test. Like HIV, the prevalence each year was relatively stable over the study period. Interestingly, 53 TPAb seroconversions were registered during the study period (937.1 PY). The median observation period per patient was 2.06 (IQR: 1.18-3.40) years. The number of infected individuals increased every year with the calculated incidence for 2014, 2015, 2016, and 2017 of 1.42, 4.00, 5.67, and 9.20/100 PY, respectively. The overall incidence of syphilis was 5.66 (95% CI: 4.32-7.40)/100 PY.

Discussion

We organized the MSM cohort in Ulaanbaatar from Dec 2013 to Dec 2017 and documented a high prevalence

(9.8%) but low incidence (0.10/100 PY) of HIV-1 infection in this cohort. The nationwide SGS surveillance conducted every two years reported the prevalence of HIV-1 infection in MSM of 0.85%, 1.8%, 10.7%, and 13.7% for 2007, 2009, 2011, and 2014, respectively (5-7,9). These data indicate that HIV-1 infection in MSM increased steeply before 2011. According to the SGS surveillance data and our data of 9.8% (Dec 2013 to Dec 2017), it could be speculated that the prevalence had reached a plateau level. The low incidence data (0.10/100 PY) adds support to our speculation. The HIV and Syphilis Surveillance Survey Report (SSR) 2017 published in 2018 also demonstrated a similar result with regard to HIV-1 prevalence in MSM 9.2% (95% CI: 8.7-9.7) (*10*). These results clearly suggest that our countermeasures for HIV-1 prevention against MSM implemented since 2010 had been effective.

Our countermeasures can be divided into two separate arms. The first was HIV-1 prevention programs for MSM and implemented since 2010 by three CBOs. Before that activity, exposure of Mongolian MSM to HIV prevention programs was limited (*11*) and coverage of HIV testing was suboptimal (*12*). Our previous molecular study documented that HIV-1 infection increased rapidly in MSM before 2011 (*8*). The population of Mongolia is around 3 million and nearly half of them live in Ulaanbaatar. Accordingly, it is assumed that the majority of Mongolian MSM live in the capital Ulaanbaatar and their social and sexual network is rather concentrated in their community. It is therefore not surprising that the spread of HIV-1 infection was rapid before 2011 in the absence of adequate knowledge about HIV-1 prevention. In contrast, the decline in infection rate was thought to be fast also when the MSM community became aware of HIV-prevention programs. The SSR 2017 report indicated that 94.4% of MSM had ever received HIV testing and 89.8% of MSM had received HIV testing in the last 12 months (*10*). It is conceivable that the HIV testing campaign by CBOs was successful. Under these circumstances, HIV-1 infected patients were disproportionally distributed. Therefore, if a cluster of infected patients were included in the cohort, the prevalence in the cohort tended to be high. However, the incidence was low and the number of newly diagnosed cases with HIV-1 infection at registration in each year was also low (Table 1). The other countermeasure was the treat-all strategy for MSM irrespective of CD4 count. The Mongolian Government implemented this strategy since early 2013, well ahead of the WHO treatment guidelines launched in 2015 recommending treat-all HIV-1 patients irrespective of CD4 count (*13*). Most of the developing countries follow WHO guidelines strictly after they were launched. Early implementation of the test and treat strategy could perhaps lead to the successful control of HIV-1 infection in MSM.

On the other hand, the prevalence of syphilis in this cohort was high (17.0%), the incidence was also high (5.66/100PY), and the numbers of newly diagnosed cases at registration over the years were also persistently high (Table 2). Furthermore, the incidence showed a trend for a gradual increase over time. With regard to the sexual behavior among MSM, the SSR reported that condom use during sexual intercourse among men increased from 44.9% in 2014 to 60.2% in 2017 (*10*). These data suggest that while sexual behaviors in Mongolian MSM might be improving, such improvement has not resulted in satisfactory control of syphilis.

This study had certain limitations. First, the number of MSM enrolled in this cohort was limited. Therefore, there was discrepancy between the high prevalence and low incidence of HIV-1 infection. It is possible we missed other expanding transmission networks, like the CRF51_01B cluster (*14*). To obtain more accurate epidemiological data, we need to include a wider range of MSM groups. However, the estimated MSM population in Mongolia is only 3,100 and about one third of those participated in this cohort (*15*). Second, nearly 40% of the MSM were tested only once, indicating a high lost-to-follow rate in this cohort, and suggests possible underestimation of the true incidence of HIV-1 infection. However, judging from the incidence of syphilis, the MSM remaining in the cohort are probably sexually active. Third, this cohort study included only MSM aged ≥ 20 of age due to the restriction imposed by the Japanese ethics regulations. However, male teenagers could be more sexually active and have limited information about HIV-1 infection. In this regard, efforts had been also directed towards the younger Mongolians (*16*). Finally, this cohort only included MSM. However, for better assessment of the epidemiology and to control HIV-1 infection in Mongolia, we need to include FSWs since the prevalence of sexually transmitted infections in this population is reported to be high (*10,17*). In this regard, our second survey documented a shift in the risk groups from MSM to heterosexual males and females (*14*). Thus, we must pay attention to the at-risk population and need more comprehensive HIV-1 prevention strategies in the future.

In conclusion, we found in the present study HIV-1 prevalence of 9.8% and HIV-1 incidence of 0.10/100 PY among MSM living in the capital of Mongolia, based on analysis of tests/data collected between 2014 and 2017. Prevention programs, such as safer sex promotion, testing campaigns, and treat-all strategy for MSM seem to contribute to the obtained data. However, the programs must be maintained from now onward, otherwise new transmission networks could appear, with a potential increase in the incidence of HIV infection.

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Dyslipidemia and cardiovascular disease in Vietnamese people with HIV on antiretroviral therapy

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Abstract: With expanding antiretroviral therapy (ART) in Vietnam, the use of second-line ART with ritonavir-boosted lopinavir (LPV/r) is increasing. However, little is known regarding the effect of LPV/r on dyslipidemia (DL) and cardiovascular disease (CVD) in people with HIV in Vietnam. A cross-sectional study was performed in a cohort of HIV-infected Vietnamese patients on ART at the National Hospital for Tropical Diseases in Hanoi, Vietnam. In addition to DL, we included hypertension (HT) and hyperglycemia (HG) as non-communicable diseases. Blood pressure, casual blood sugar levels, and the lipid profile were evaluated cross-sectionally in October and November 2016. The incidence of CVD was calculated in the cohort. We determined factors associated with diseases by univariate and multivariate analyses. A total of 1,346 subjects were evaluated for their non-communicable diseases. The subjects' mean age was 39.2 years and 41.8% were women. A total of 10.5% of the subjects had exposure to LPV/r. DL, HT, and HG was diagnosed in 53.5%, 24.4%, and 0.8% of the subjects, respectively. In multivariate analysis, age (OR = 1.040; 95% CI, 1.025-1.055), female sex (OR = 0.335; 95% CI, 0.264-0.424), and LPV/r exposure (OR = 3.251; 95% CI, 2.030-5.207) were significantly associated with DL. The incidence rate of CVD was 1.87/1,000 person-years (15 incidental cases in 8,013 person-years). LPV/r exposure was not a risk factor for the incidence of CVD. Although a causative relation with LPV/r and CVD was not identified in this study, attention should be paid to CVD for patients on LPV/r in the future.

***Keywords*:** dyslipidemia, cardiovascular disease, human immunodeficiency virus, Vietnamese, lopinavir-boosted ritonavir

Introduction

Cardiovascular disease (CVD) is becoming one of the major comorbidities in human immunodeficiency virus (HIV)-infected patients since widespread use of antiretroviral therapy (ART) has decreased AIDS-associated mortality (*1-3*). Some classes of antiretroviral drugs, such as protease inhibitors, can cause dyslipidemia (DL), which might lead to CVD (*4-7*). Among protease inhibitors, ritonavir-boosted lopinavir (LPV/r) is still a commonly used antiretroviral drug that World Health Organization guidelines recommend as the second-line salvage regimen in Vietnam (*8*). Lopinavir requires 200 mg of ritonavir as a booster and affects the lipid profile among various protease inhibitors. Recent studies have suggested that use of LPV/r is especially associated with CVD (*9,10*). The period of exposure to LPV/r has been increasing because of long-term survival of people living with HIV. Furthermore, there are situations that do not allow substitution of protease inhibitors to integrase inhibitors, which do not affect the lipid profile, in resource-limited settings because of budget limitations. The long-term effect of LPV/r on DL and CVD is of concern in this context.

Therefore, we conducted a cross-sectional study to evaluate the prevalence of DL and its associated factors. Furthermore, we performed a review of this cohort to estimate the incidence rate of CVD and causative relation between CVD and exposure to LPV/r in Vietnamese people living with HIV.

Materials and Methods

Study design

This study consisted of two parts, and was performed at the National Hospital for Tropical Disease, Hanoi, Vietnam. This hospital is one of the largest out-patient clinics for people with HIV in Vietnam. The study

population included Vietnamese people with HIV aged older than 17 years. In the first part of the study, we conducted a cross-sectional study to evaluate the prevalence of non-communicable diseases, including DL, hyperglycemia (HG), and hypertension (HT) and their associated factors in an observational, single-center cohort of Vietnamese HIV-infected patients in the National Hospital for Tropical Disease in October and November 2016. In the second part of the study, we reviewed the incidence of CVD for all participants of a prospective cohort since its establishment to April 2017. The participants of the cohort visited the hospital every 6 months and follow-up period and the incidence rate were evaluated.

The study was approved by the Human Research Ethics Committee of the National Hospital for Tropical Disease and Hanoi City, Hanoi. All patients who were recruited in the study provided written informed consent for their clinical and laboratory data to be used and published for study purposes. The study was performed according to the principles expressed in the Declaration of Helsinki.

Measurements

Data collection of systolic and diastolic blood pressure (mmHg), casual blood sugar levels (mg/dL), and the lipid profile (triglycerides [TG], total cholesterol, and high-density lipoprotein [HDL] [mg/dL]) was performed in October and November 2016 for every patient who was registered in the cohort. Other data included the following: demographic variables (height, weight, sex, and age); a complete history of ART; a history of smoking; a history of CVD including stroke, congestive heart failure, and coronary artery disease; use of drugs for prophylaxis against opportunistic infections; CD4 cell counts (cells/mm3, measured by flow cytometry); and plasma HIV-RNA (copies/mL, measured using the Roche Cobas Taqman analyzer; Roche Molecular Diagnostics, Pleasanton, CA). Data were collected every 6 months until October 2017. Dyslipidemia was defined as TG levels > 150, HDL levels < 40, or c-LDL levels > 140 mg/dL (c-LDL was calculated as total cholesterol−HDL−TG/5). Hypertension was defined as systolic pressure > 140 mmHg or diastolic pressure > 90. HG was defined as casual blood glucose levels > 200 mg/dL. The incidence rate of CVD was calculated as the number of CVD cases divided by person-years observed in the cohort from the time of enrollment of the cohort to April 2017.

Statistical analysis

Statistical analysis was performed for descriptive data (mean and standard deviation), and univariate and multivariate analyses. Absolute and relative frequencies were used for continuous and categorical variables, respectively. To evaluate the association between exposure to LPV/r and other categorical variables, the chi-square or Fisher's exact test was applied as required. The independent t test or one-way ANOVA was used to compare means, and in case of asymmetry, the Mann-Whitney or Kruskal-Wallis test was also used.

Variables that were significantly associated with non-communicable diseases and CVD in univariate analysis were included in multivariate analysis. Logistic regression was used to determine the factors associated with non-communicable diseases in univariate and multivariate analyses. Cox proportional hazards regression was used to determine the factors associated with the incidence of CVD in univariate and multivariate analyses.

Statistical significance was defined as a two-sided p value < 0.05. We used odds ratios (ORs) and 95% confidence intervals (95% CIs) to estimate the association of each variable with non-communicable diseases. All statistical analyses were performed with SPSS ver. 25.0 (IBM SPSS, Chicago, IL).

Results

Table 1 shows the baseline characteristics of the subjects for the cross-sectional study. A total of 1,346 Vietnamese people with HIV were evaluated for the cross-sectional study. The subjects' mean age was 39.2 years and 41.8% of the patients were women. The mean body weight and body mass index were 56.2 kg and 21.4 kg/m^2, respectively, which represented a population with a low body weight. A total of 142 (10.5%) subjects were exposed to LPV/r. Subjects with LPV/r exposure had significantly higher serum creatinine, TG, and non-HDL cholesterol, and lower HDL levels compared with those without LPV/r exposure. However, there were no significant differences in basic demographics, including age, sex, and body weight, between subjects with and those without LPV/r exposure.

The prevalence of each non-communicable disease is shown in Table 2. The prevalence of DL, HT, and diabetes mellitus was 53.5%, 24.4%, and 24.4%, respectively. Of the subjects with DL, 669 (92.9%) subjects had hypertriglyceridemia. Tables 3, 4, and 5 show factors that were associated with DL, HT, and diabetes mellitus in univariate and multivariate analyses, respectively. Age (OR = 1.040; 95% CI, 1.025-1.055), female sex (OR = 0.335; 95% CI, 0.264-0.424), and LPV/r exposure (OR = 3.251; 95% CI, 2.030-5.207) were significantly associated with DL in multivariate analysis (Table 3). Older age was significantly associated with HG and HT in multivariate analysis (Tables 4 and 5). Exposure to LPV/r was significantly associated with HT in a protective manner in multivariate analysis (Table 5), which reflected lower diastolic blood pressure in subjects with LPV/r exposure.

Table 1. Baseline characteristics of Vietnamese patients with HIV on ART (n = 1,346)

Variables	LPV/r exposure-	LPV/r exposure+	Entire group	p value
Number of patients	1,204	142	1,346	
Age, years	39.2 ± 8.84	39.2 ± 8.57	39.2 ± 8.81	1.000
Female, *n* (%)	502 (41.7%)	61 (43.0%)	563 (41.8%)	0.773
Body weight, kg	56.3 ± 8.66	55.0 ± 8.83	56.2 ± 8.68	0.114
BMI, kg/m^2	21.4 ± 2.5	21.2 ± 2.8	21.4 ± 2.6	0.468
CD4+ count, /mL	483.1±197.9	475.7±212.4	482.3 ± 199.4	0.676
HIV RNA < 400 copies/mL	1180 (98.0%)	135 (95.1%)	1315 (97.8%)	0.032
Serum creatinine, mg/dL	0.85 ± 0.21	0.94 ± 0.23	0 .87 ± 0.22	< 0.001
Blood glucose, mg/dL	92.7 ± 20.6	94.4 ± 27.6	92.8 ± 21.5	0.384
Systolic blood pressure, mmHg	118.0 ± 15.5	117.6 ± 14.0	118.0 ± 15.3	0.739
Diastolic blood pressure, mmHg	75.5 ± 11.9	72.7 ± 10.5	75.2 ± 11.8	0.006
TG, mg/dL	192.2 ± 286.9	351.9 ± 553.5	209.0 ± 256.5	< 0.001
HDL, mg/dL	58.9 ± 23.1	51.5 ± 15.1	58.1 ± 22.5	< 0.001
non-HDL cholesterol, mg/dL	115.3 ± 46.6	138.8 ± 71.4	117.9 ± 50.3	< 0.001
Time from diagnosis of HIV infection, years	6.5 ± 3.8	10.3 ± 4.2	6.9 ± 4.1	< 0.001
Time from ART initiation, years	5.1 ± 3.0	8.6 ± 3.3	5.5 ± 3.2	< 0.001
Current smoking	283 (23.5%)	25 (17.6%)	308 (22.9%)	0.113

Data are expressed as mean ± SD or *n* (%). ART: antiretroviral therapy; HDL: high-density lipoprotein; LPV/r: ritonavir-boosted lopinavir; TG: triglycerides.

Table 2. Prevalence of non-communicable diseases in Vietnamese patients with HIV on ART (n = 1,346)

Non-communicable diseases	*n* (%)
Obesity	108 (8.0)
Hypertension (HT)	329 (24.4)
Hyperglycemia (HG)	11 (0.8)
Dyslipidemia (DL)	720 (53.5)
TG ≥ 150 mg/dL	669 (49.7)
HDL ≤ 39 mg/dL	209 (15.5)
Non-HDL cholesterol ≥ 210 mg/dL	47 (3.5)

Data are expressed as *n* (%). HDL: high-density lipoprotein; TG: triglycerides.

With regard to the incidence rate of CVD, 15 CVD events (incidence rate of CVD: 1.87/1,000 person-years) occurred in the cohort during the study period, with a mean follow-up period of 4.6 years and 8,013 person-years. Of the 15 CVD cases, two cases were coronary artery disease, 11 cases were stroke, and one case was congestive heart failure. In the Cox proportional hazard model, exposure to LPV/r was not significantly associated with the incidence of CVD (hazard ratio = 1.417; 95% CI, 0.446-4.498; p = 0.554). Age was the only factor that was associated with the incidence of CVD (hazard ratio = 1.123; 95% CI, 1.075-1.172; p < 0.001). Other non-communicable diseases, exposure of abacavir, and a history of smoking were not associated with the incidence of CVD.

Discussion

We evaluated the prevalence of non-communicable diseases and their associated factors among well-controlled Vietnamese people with HIV on ART. The prevalence of DL was 53.5%, which was disproportionally high compared with that of HG (0.8%) and HT (24.4%). LPV/r was strongly associated with DL in addition to known risk factors. However, exposure to LPV/r was not associated with the incidence of CVD. This could be partly attributable to underestimation of the incidence of CVD because a considerable number of the cause of deaths in this cohort remains unknown. Importantly, this is the first study that not only showed that exposure to LPV/r was strongly associated with DL, but it also estimated the incidence rate of CVD and its risk factors among Vietnamese people with HIV on ART.

Protease inhibitors affect the lipid profile and may cause CVD. Previous studies have suggested that using LPV/r can enhance renal toxicity of tenofovir and LPV/r is associated with renal dysfunction (*11,12*). Depending on the situation, substitution of other protease inhibitors, such as ritonavir-boosted darunavir or integrase inhibitors, for LPV/r can be used as salvage regimens (*13*). If this is not possible because of budget limitations, lipid-lowering therapy can be beneficial in people who are taking LPV/r with known risk factors, including older age, HT, and diabetes mellitus (*14-18*).

Our study has several limitations. In this prospective cohort, information on the cause of death or reason of loss to follow-up was partially missing. This could have led to underestimation of the incidence of CVD as mentioned above. Furthermore, some cases of CVD occurred before the timing of our cross-sectional study, which failed to reflect a causative relation between CVD and non-communicable diseases. In fact, non-communicable diseases, which are known as risk factors, were not significantly associated with the incidence of CVD in our study. Related to this limitation, information about treatment for non-communicable diseases, including lipid-lowering therapy, was not reflected in this analysis. This could have led to underestimation of

Table 3. Associated factors for dyslipidemia (DL) as estimated by univariate and multivariate analyses (n = 1,346)

Variables	Univariate analysis		Multivariate analysis		p value
	OR	95% CI	OR	95% CI	
Age per year-increase	1.053	1.039-1.068	1.040	1.025-1.055	< 0.001
Female	0.323	0.258-0.404	0.335	0.264-0.424	< 0.001
BMI per 1 kg/m^2-decrement	1.193	1.140-1.249			
CD4+ cell count per cell/μL	1.101	0.999-1.001			
HIV RNA > 400 copies/mL	0.755	0.365-1.559			
Time from diagnosis of HIV infection per year-increase	1.072	1.043-1.102	1.007	0.957-1.059	0.799
Time from initiation of ART per year-increase	1.123	1.085-1.163	1.065	0.998-1.138	0.059
Exposure of LPVr	3.347	2.222-5.042	3.251	2.030-5.207	< 0.001
Exposure of TDF	0.998	0.763-1.305			
Exposure of ABC	2.033	1.075-3.846	0.769	0.357-1.660	0.504

ABC: abacavir; ART: antiretroviral therapy; CI: confidence interval; LPV/r: ritonavir-boosted lopinavir; OR: odds ratio; TDF: tenofovir disoproxil fumarate.

Table 4. Associated factors for hyperglycemia (HG) as estimated by univariate and multivariate analyses (n = 1,346)

Variables	Univariate analysis		Multivariate analysis		p value
	OR	95% CI	OR	95% CI	
Age per year-increase	1.082	1.029-1.137	1.075	1.017 – 1.136	0.011
Female	0.519	0.137-1.965			
BMI per 1 kg/m^2-decrement	1.149	0.932-1.417			
CD4+ cell count per cell/μL	1.002	0.999-1.004			
HIV RNA > 400 copies/mL	0.000	0.000–			
Time from diagnosis of HIV infection per year-increase	1.160	1.018-1.321	0.829	0.541-1.271	0.390
Time from initiation of ART per year-increase	1.337	1.119-1.598	1.542	0.952-2.500	0.079
Exposure of LPVr	3.227	0.846-12.304			
Exposure of TDF	0.664	0.175-2.518			
Exposure of ABC	6.520	1.368-31.071	2.764	0.466-16.387	0.263

ABC: abacavir; ART: antiretroviral therapy; CI: confidence interval; LPV/r: ritonavir-boosted lopinavir; OR: odds ratio; TDF: tenofovir disoproxil fumarate.

Table 5. Associated factors for hypertension (HT) as estimated by univariate and multivariate analyses (n = 1,346)

Variables	Univariate analysis		Multivariate analysis		p value
	OR	95% CI	OR	95% CI	
Age per year-increase	1.061	1.046-1.076	1.051	1.035-1.067	< 0.001
Female	0.363	0.274-0.479	0.432	0.322-0.577	< 0.001
BMI per 1 kg/m^2-decrement	1.155	1.100-1.213	1.119	1.063-1.177	< 0.001
CD4+ cell count per cell/μL	0.999	0.999-1.000	1.000	0.999-1.000	0.535
HIV RNA > 400 copies/mL	0.939	0.399-2.208			
Time from diagnosis of HIV infection per year-increase	1.001	0.971-1.032			
Time from initiation of ART per year-increase	1.035	0.996-1.075			
Exposure of LPVr	0.633	0.403-0.993	0.623	0.390-0.996	0.048
Exposure of TDF	0.854	0.419-1.741			
Exposure of ABC	0.854	0.419-1.741			

ABC: abacavir; ART: antiretroviral therapy; CI: confidence interval; LPV/r: ritonavir-boosted lopinavir; OR: odds ratio; TDF: tenofovir disoproxil fumarate.

the prevalence of non-communicable diseases. Although this study did not identify LPV/r as a risk factor for the incidence of CVD, attention should be paid to preventing the incidence of CVD in people who are taking LPV/r with known risk factors.

In conclusion, the present study shows a high prevalence of DL in Vietnamese people with HIV and exposure to LPV/r is strongly associated with DL, in addition to other known risk factors. Attention to CVD is necessary for patients on LPV/r in consideration of their aging in the future.

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Conflicts of interest

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Infectious Disease Emergency Specialist (IDES) Training Program in Japan: an innovative governmental challenge to respond to global public health emergencies

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Abstract: In 2015, Japan created a unique governmental program to train experts in health emergencies called Infectious Disease Emergency Specialist (IDES). This is a concept paper to set out the goal and structure of the program, and to describe the achievement and the way forward to further contribute to global health security. The IDES program background, mission, structure, achievement, and future directions were reviewed and discussed by the IDES trainees, graduates, and program coordinators/supervisors. Since 2015, thirteen Japanese medical doctors have graduated from the program while five are currently in training. The IDES core competencies were identified in the context of a wide range of skillsets required for health emergencies. A large national and global network has been created through the training. Coordinated work with surge capacity of experts is of paramount importance to prepare for and respond to public health emergencies. The IDES program can be a good model to many other governments, and contribute to global health security.

***Keywords*:** health emergencies, emergency preparedness, outbreak response, public health, global health, health security

Introduction

Health emergencies not only pose public health threat to a country, but also jeopardize human health security at the international level. The 2014 Ebola virus disease outbreak in West Africa revealed how epidemic-prone infectious diseases can endanger global health security (*1*). The Government of Japan deployed a total of twenty Japanese experts to the affected countries through the World Health Organization (WHO) in response to the outbreak (*2*). However, the government of Japan was unable to dispatch a sufficient number of specialists familiar with public health emergency due to such infectious diseases, in a coordinated manner with global partners. A question was raised: how can the government accumulate and utilize individual experience and expertise as a national asset in the setting of health emergencies?

In order to further strengthen Japan's response to future outbreaks, Japan's Ministry of Health, Labour and Welfare (MHLW) launched a unique training program called Infectious Disease Emergency Specialist (IDES) training program in October 2015 (*3*). It is unique in that the program is governmental, organized by MHLW, involving various national institutes with different expertise in health emergencies. As of October 2019, thirteen Japanese medical doctors completed the program, and five are currently in the training.

The aim of this article is to present the IDES training program and to explore the possibility of Japan's further contribution to global health security as a global partner.

Core competencies and program structure

The mission of the IDES training program is to enhance Japan's contribution to global health security through capacity development of Japanese medical experts in health emergencies. Upon completion of the training, the graduates are expected to be able to respond to health emergencies at both national and international levels in collaboration with global partners. Thus, medical doctors with diverse backgrounds, who are not only infectious disease specialists, but also pediatricians, general internists, obstetricians, and public health practitioners, have been selected as IDES trainees. The training program gives a unique opportunity to participate in various on-the-job trainings both domestically and internationally, while pre-existing programs, such as Field Epidemiology Training Program (FETP) in Japan, provide training mainly in Japan and focus on a particular aspect of public health such as epidemiology (*4*).

The core competencies the IDES trainees should acquire through the program are listed in Table 1. The two-year program is composed of mainly two parts: the domestic public health training in the first year and the overseas training in the second year (Figure 1). During the first year, IDES trainees are involved in four major components: national health policy management at the Infectious Diseases Control Division of MHLW; clinical implication of national health policy at the National Center for Global Health and Medicine (NCGM), which is a national hub to respond to infectious diseases designated by the Infectious Disease Control Law; field epidemiological work such as outbreak response and disaster response with mutual interactions with the FETP program at the National Institute of Infectious Diseases (NIID); and quarantine activities at major international airports and seaports, aiming to better understand International Health Regulations (IHR) (Figure 1). IDES trainees can also receive elective training, such as basic laboratory training and/or biorisk management at the biosafety level (BSL) 4 facility

Table 1. Core competencies to be acquired by Infectious Disease Emergency Specialist (IDES) training program in Japan

Core competencies
i) Understanding and practical application of infectious diseases and epidemiology
ii) Collection, analysis, interpretation and dissemination of information on epidemic-prone infectious diseases
iii) Strong work ethics and self-discipline to work under difficult circumstances
iv) Leadership
v) Team building and management
vi) Coordination and communication skills

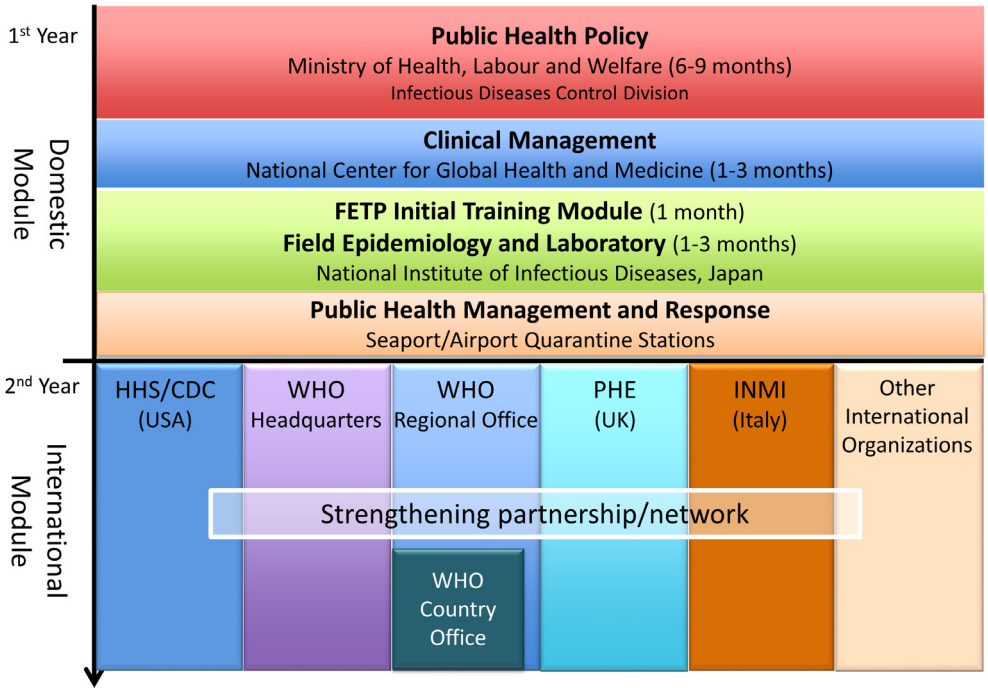

Figure 1. A model curriculum of Infectious Disease Emergency Specialist (IDES) training program in Japan.

at the NIID, or research training related to health emergency and preparedness at the National Institute of Public Health (NIPH). The first-year training is designed flexibly so that the trainees further strengthen their expertise and develop competencies, and they start to formulate their roles in health emergency response in Japan and the world.

The second-year oversea training gives further opportunities to strengthen the above knowledge and skill sets to become a well-balanced and competent expert in more global settings, leading to expand network and collaboration with other major global partners. As of October 2019, the trainees have been dispatched to the following international and national organizations abroad: WHO headquarters (Switzerland); WHO Western Pacific Regional Office (WPRO) (the Philippines); Global Alliance for Vaccines and Immunizations (Gavi) (Switzerland); Department of Health and Human Services (HHS) (United States of America (USA)); Centers for Disease Control and Prevention (CDC) (USA); Public Health England (United Kingdom) and National Institute for Infectious Diseases "Lazzaro Spallanzani" (Italy). The secondment is determined by individual expertise and interest, and his/her future contribution to Japan. The strategic planning to maximize capacity development for the Government is also considered. For example, some trainees delve into operational management and/or preparedness for chemical, biological, radiological, nuclear, and explosive (CBRNE) threats and natural disasters, based on "all-hazard preparedness and response approach" at a national level (*5*). Meanwhile, others receive more clinically-oriented training, including patient management of infectious diseases rarely seen in Japan, and infection prevention and control at the health facility level.

Responsibilities and contribution after training

After the training, IDES graduates have a wide range of career paths. They further develop their own expertise in their own affiliated organization. Some work in the area of research such as epidemiology while others work as a clinician to prepare for and respond to epidemic-prone infectious diseases. They also have a chance to work at governmental offices such as MHLW and quarantine stations.

MHLW constantly shares relevant information with IDES graduates. In case of health emergencies that require timely and upscale response, MHLW asks them for technical support. Where appropriate, MHLW dispatches IDES graduates to the affected area. The scope of future work at MHLW also includes further integration of IDES into other existing health emergency schemes at the national level such as Japan Disaster Relief (JDR) Infectious Diseases Response Team, a health emergency team organized by Japan International Cooperation Agency (JICA) and Ministry of Foreign Affairs (*6*), and collaboration with global response schemes such as Global Outbreak Alert and Response Network (GOARN) coordinated by WHO (*7*). In July 2018 and August 2019, four IDES graduates in total were actually dispatched to Bangladesh for a diphtheria outbreak in Cox's Bazar, and to Democratic Republic of the Congo for the on-going Ebola outbreak, respectively. The former dispatch was closely coordinated with the GOARN. Additionally, IDES will be involved in preparedness for and management of mass-gathering events, especially the Olympic and Paralympic Games in Tokyo in 2020.

Future of IDES

The IDES training program provides unique opportunities with flexibility. One of the strengths of this program is that the trainees get a lot of opportunities to work at global public health agencies: building a network with a wide range of global partners will greatly contribute to subsequent activities the government gets involved with, even in peacetime. The trainees also have a chance to strengthen their own skill set to contribute at a national and international level, while the program helps the Government of Japan improve response capacity for health emergencies. IDES trainees and graduates with diverse backgrounds and competencies can develop a close network to discuss health emergencies constantly and to further engage in operational management and field activities during health emergency events.

However, opportunities for improvement exist. First, a more precise answer would be required to determine to what extent and what type of expertise is particularly required to fill the gaps in outbreak response and preparedness at the national level through the IDES training program. In addition, opportunities for continuous education should be ensured to update knowledge and skills, leading to maintain their motivation and expertise for future response. Lastly, financial resources for IDES trainees and graduates to fulfill such changing demands at a national and international level need to be secured.

Conclusion

Global public health threats due to infectious diseases can occur anytime, anywhere in the world. Epidemic-prone infectious diseases spread more rapidly and widely, given the mobility of humans, urbanization, the climate change and the associated change in animal and environmental ecology (*8*). Also, poverty, conflict, and natural or man-made disasters make certain populations vulnerable to such infectious diseases. Thus, appropriate preparedness for and response to infectious diseases are the cornerstone for current global health security (*9*), and such capacities and capabilities should be built

at the national, regional and global level (*10*). While United Nations (UN) sets universal health coverage (UHC) as a goal of Sustainable Development Goal (SDG) 3, Japan has been a strong advocate for UHC (*11*). Work on health emergencies and UHC are two sides of the same coin: strengthening capacity for health emergencies leads to achieve UHC. In order to achieve such effective and sustainable capacity development, properly trained personnel with knowledge and skills is as important as having medical stockpiles. MHLW continues to refine the IDES training program, and further commits to global health security, collaborating with global partners.

Statement The views expressed in the manuscript are the authors' own and not an official position of the institutions.

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The cooperation between professional societies contributes to the capacity building and system development for prevention and control of cancer in low- and middle-income countries: the practice of Cervical Cancer Prevention and Control Project in Cambodia

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Abstract: Globally, an estimated 570,000 women are newly diagnosed with cervical cancer, and 311,000 women die every year, with approximately 90% of the cases occurring in low- and middle-income countries (LMICs). Cervical cancer is the most common cancer in women in Cambodia, with age-standard incidence rate of 13.5/100,000 and mortality rate of 10.1/100,000. This paper introduces the educational and managerial interventions of Cambodia Cervical Cancer Project 2015-2018 by two professional societies of Cambodia and Japan. It can be categorized into three phases: health education and screening; diagnosis and treatment of precancerous lesions; and pathology service. Human papillomavirus test-based cancer screening and treatment of precancerous lesions were successfully initiated. Key factors contributed to optimal outcomes are partnership between two professional societies with strong commitment, and a comprehensive and stepwise quality-focused approach. A complementary role and joint society initiatives is a novel approach and substantial in sustainability for developing a system of cervical cancer management. This effort might serve as a good example how professional societies can contribute to capacity building and system development for prevention and control of cancer in LMICs.

***Keywords*:** cervical cancer, screening and treatment, human papillomavirus test, professional society

Introduction

Globally, an estimated 570,000 women are newly diagnosed with cervical cancer, and 311,000 women die every year, with approximately 90% of the cases occurring in low- and middle-income countries (LMICs) (*1*). Since the main cause of cervical cancer is the persistent infection of high-risk subtypes of human papillomavirus (HPV), this cancer is largely preventable through primary prevention with the HPV vaccine and health education as well as secondary prevention with screening and treatment of precancerous lesions (*2*). These effective primary and secondary prevention measures are still not accessible to the majority of women in LMICs, and cervical cancer is identified only at an advanced stage, resulting in a high associated rate of death in these countries.

In Cambodia, with a female population of 8.2 million, cervical cancer is the most common cancer in women. The age-standard incidence rate (13.5 per 100,000 women) and mortality rate (10.1 per 100,000 women) are much higher than regional and global estimates (*3*). With an urgent need for action, cervical cancer was elevated to a disease to be given priority in the National Strategy for the Prevention and Control of Non-communicable Diseases (NCDs) 2007-2010 (*4*). In 2008, the Cambodian Ministry of Health (MOH) issued a national guideline for cervical cancer screening with visual inspection with acetic acid (VIA) and immediate treatment with cryotherapy (*i.e.*, "screen and treat") as the nationwide strategy, followed by the national strategic plan for NCDs 2013-2020 (*5*).

Despite these efforts, the "screen and treat" strategy was still barely accessible to most women in Cambodia in 2015. Pilot programs were run with the support of different nongovernment organizations (NGOs), where

VIA was used for screening at clinics and health centers, with the referral of positive screens to district hospitals for cryotherapy (*6*). None of the programs were successful in scaling up due to the lack of collaboration in harmonizing clinical practice. Recently, the HPV test has become more affordable with its higher accuracy and more objective endpoint than other screening methods (*i.e*., VIA or cytology) (*7*). However, its implementation is observed only in a few resource-limited countries (*8*).

The Cambodian Society of Gynecology and Obstetrics (SCGO) is the only society of professional obstetricians and gynecologists in Cambodia, established in 1997, with approximately 180 members as of 2015. With a request from the MOH, the SCGO was eager to address the issue of limited access to screening and treatment but needed technical support. The SCGO and the Japan Society of Obstetrics and Gynecology (JSOG) initiated a collaborative project – Cambodia Cervical Cancer Project- in 2015.

In this paper, we introduce the educational and managerial interventions of Cambodia Cervical Cancer Project conducted by the SCGO and JSOG. This may serve as a good example of how professional societies can contribute to capacity building and system development for prevention and control of cancer in LMICs.

Key interventions and primary outcomes of Cambodia Cervical Cancer Project

This project was designed based on the WHO's guide to Comprehensive Cervical Cancer Control (*2*). It can be categorized into three project phases. Phase A (2015-2018) aimed to raise awareness of women's health and access to cervical cancer screening among factory workers; phase B (2015-2018) aimed to improve gynecologic capacity for the diagnosis and treatment of precancerous lesions; and phase C (2017-present) aimed to strengthen the pathological capacity for cancer diagnosis.

Table 1 summarizes the characteristics of each phase, and presents key educational and managerial interventions conducted at the individual, organizational, and systematic levels.

Phase A (Health education and screening)

Factory workers were selected for the target population because of the rapid increase in the number and social demand for their health and welfare in Cambodia (*9*). To assess baseline knowledge, attitude, and practice of the target population regarding reproductive health and cervical cancer, a cross-sectional survey was conducted in Factory A in March 2016. Among 443 female workers, their level of knowledge of women's health and cervical cancer was low. Approximately 85% reported that their sources of information were relatives or friends, indicating that they rarely have opportunities to receive accurate knowledge from health professionals (*10*). Based on their educational needs, teaching materials on cervical cancer were developed.

The first health education session was held at Factory A in August 2016. Through sharing of the activities among factories in the Special Economic Zone, health education was expanded to other factories. The educational contents ultimately included five themes: basic hygiene, women's health, birth spacing, care during pregnancy and cervical cancer.

In parallel with health education, cervical cancer screening was offered on site to minimize the geographic access barrier to screening. The HPV test (careHPV®, QIAGEN) was used because of its higher accuracy, more objective endpoint, and limited pathological capacity in the country (*7,11*). With no reliable data on HPV infection in Cambodia, the eligibility criteria for screening were determined to be those who attended the health education program, who were over age 25 years old, who had ever had a sexual partner and who were not pregnant. Informed consent was obtained before the screening. Participants received group counseling after screening on the follow-up procedure as needed.

The collected cervical samples were sent to one of the national hospital laboratories where trained technicians performed the HPV assay. They became competent handling the HPV assay after one-month training with sufficient proficiency confirmed by a control laboratory in Japan (*12*). For women who tested positive, considering the risk of overtriage (*13*), a follow-up examination was scheduled at one of the target hospitals for Phase B.

Between August 2016 and May 2018, health education was conducted 14 times at five factories by a mobile team of midwives, SCGO gynecologists and SCGO secretariat staff. A total of 2,597 workers, including men, participated, and 687 women were eligible for screening. A mobile cervical cancer screening was conducted for the first time in Cambodia in the economic zone in June 2017, with a second round in April 2018. Among eligible women, 132 participated (screening rate 19.2%) in two rounds of screening, and 15 had positive HPV results (HPV positivity rate 11.4%).

Phase B (Diagnosis and treatment of precancerous lesions)

Situational analysis to examine the current practice at the target hospitals in November 2015 found that machines for diagnosis (colposcopy) and treatment of precancerous lesions (loop electrosurgical excision procedures (LEEP)) were already installed in all three hospitals but were often used improperly. Records regarding the number of abnormal cases detected and treated were poorly recorded (*14*).

To improve the skill of colposcopy and LEEP, a

Table 1. Characteristics and a summary of interventions in three project phases

Project Phase	Phase A (Health education and screening)	Phase B (Diagnosis and treatment of precancerous lesions)	Phase C (Pathology service)
Objective	Raise awareness of women's health and access to cervical cancer screening	Improve gynecologic capacity for diagnosis and treatment of precancer lesions	Strengthen pathological capacity for cancer diagnosis
Period	2015-2018	2015-2018	2017-present
Targets			
Individual level	Factory workers and managers	Gynecologists and laboratory technicians	Pathologists and pathology technicians
Organizational level	Five PPSEZ-based factories	Three national hospitals	Three national hospitals and one national hospital
Funding agency[a]	JICA, MHLW	JICA, MHLW	MHLW
Implementing organizations	SCGO, JSOG, NCGM	SCGO, JSOG, NCGM	SCGO, JSCC, NCGM
Budget	Total USD 534,000 for 3 years for phase A and phase B		USD 90,000/year
Interventions at individual level	<Factory workers> ▪ KAP survey on women's health ▪ Health education on women's health ▪ Cervical cancer screening, early diagnosis and treatment (as needed) <Factory managers> ▪ Advocacy for raising awareness on cervical cancer and women's health ▪ Feedback on the results of the programs ▪ Discussions on ways to maintain healthy workforce	<Gynecologists> ▪ Training on the management of cervical cancer screening program (notice-screening-reporting-monitoring) ▪ Technical training on the collection of cervical sample for HPV testing ▪ Technical training on diagnosis by colposcopy and treatment by LEEP ▪ On-site training for implementation of standard protocols <Laboratory technicians> ▪ Technical training on the use of HPV testing platform	<Pathologists> ▪ Lectures on pathological diagnosis ▪ Discussions on ways to strengthen pathology service in Cambodia (establishment of a society, training of technicians, *etc*.) ▪ Provision of opportunities to be trained abroad to learn how well-developed pathology service and educational system could look like <Pathology technicians> ▪ Technical training on preparation of quality pathology slides
Interventions at organizational level	<PPSEZ Secretariat> ▪ Reporting of the activities and outcomes of the KAP survey, health education and cervical cancer screening programs <Target factories> ▪ Provision of educational training to the medical office staff	<Target hospitals> ▪ Provision of LEEP instruments ▪ Provision of equipment and supplies for HPV testing ▪ Development of hospital-level cervical cancer registry ▪ Implementation of bar-code system for lab sample management	<Target hospitals> ▪ Promotion of good working environment (ventilation, temperature management, *etc*.) ▪ Development of standard operation procedure for pathology slide preparation
Interventions at network/policy level	▪ Development of teaching materials and brochures on cervical cancer ▪ Development of a health education program on cervical cancer and women's health ▪ Sharing of the program activities to other PPSEZ-based factories at regular meetings ▪ Sharing of program activities to a wider audience through SCGO website and SNS	▪ Development of protocols for cervical cancer screening ▪ Development of protocols and a reference book for early diagnosis and treatment of cervical cancer ▪ Provision of continuing professional development through annual SGCO and JSOG conferences, seminars, lectures, and clinico-pathological conferences ▪ Reporting of the activities and outcomes of the project to the Ministry of Health	▪ Educational support of pathology post-graduate residency program ▪ Provision of continuing professional development through clinico-pathological conferences

[a] Exchange rate for calculation: 1 USD = 110 JPY. JICA: Japan International Cooperation Agency; JSCC: Japanese Society of Clinical Cytology; JSOG: Japan Society of Obstetrics and Gynecology; MHLW: Japanese Ministry of Health, Labour and Welfare; NCGM: National Center for Global Health and Medicine, Japan; PPSEZ: Phnom Penh Special Economic Zone; SCGO: Cambodian Society of Gynecology and Obstetrics; KAP: knowledge, attitudes, and practices; LEEP: Loop Electrosurgical Excision Procedure; SNS: social networking services.

series of hands-on trainings was conducted both on-site and in Japan between 2015 and 2018. LEEP was favored over cryotherapy for the treatment of precancerous lesions because it allows the lesions to be pathologically examined for definitive diagnosis and improves surgical capacity at tertiary hospitals responsible for cancer care. To harmonize practice, a standard clinical protocol was developed to guide clinical decision-making, and it was reviewed and revised after a year. Reporting forms and a registry for HPV-positive cases were also developed for monitoring.

In total, 13 physicians from three national hospitals were trained to acquire adequate skills in colposcopy and LEEP by 39 JSOG member physicians from 14 universities. Among the four who screened positive from the first round of screening in June 2017, none came

for follow-up appointments. In addition to discussion with factory managers to allow sick leave, a call-and-recall system was introduced in the second round in April 2018, where SCGO secretarial staff made calls to those who screened positive to remind them to come for a follow-up and to respond to any concerns they had (*15*). The participation rate increased to 54.5% (six out of 11). Project outcomes were shared with the MOH, society members, WHO and NGOs in the dissemination seminar in September 2018. HPV test-based screening was recognized as a possible option in Cambodia.

Phase C (Pathology service)

Through phase B, the obstacle to scaling up a cervical cancer screening program in Cambodia was identified as an extremely limited capacity for pathological services. When any screening program is scaled up in a country, there will inevitably be a large increase in the detection rate of cervical cancer. In 2017, however, there were only four pathologists and 15 pathology technicians actively working in the whole country. The situation was even worse than in 2014 because of the retirement of older generations (*16*).

At the target hospitals, although basic pathology equipment and supplies were available, slides were often difficult to read due to inadequate preparation. Step-by-step training was offered to technicians for quality slide preparation. Five pathology residents, enrolled in the national residency program that started in 2015 to increase the number of pathologists, were also trained for their capacity-building in diagnosis and quality management (*17*).

Under the initiative of the SCGO, a clinico-pathological conference (CPC) was introduced to improve case management (*17*). In the beginning, pathologists and gynecologists tended to criticize each other on discrepancies between clinical and pathological diagnoses, but with close mentoring by Japanese physicians, a mutual learning environment was soon created. The CPC has gradually become a routine practice for developing clinical management skills.

The cooperation between professional societies contributes to capacity building and system development for cervical cancer prevention and control in Cambodia

Factors that contributed to optimal outcomes within a relatively small budget were identified from a series of focus group discussions and in-depth interviews during the project. Main factors are the partnership between two professional societies with strong ownership and commitment by the SCGO; and a comprehensive and stepwise quality-focused approach with stakeholder involvement

Since 1998, JSOG members have been working with the Cambodian MOH through development projects. Individual collaboration became more institutional in 2012 when an exchange program was initiated between the JSOG and SCGO. The idea for this project came from the SCGO. Throughout the project implementation, the JSOG respected the initiative of the SCGO and adopted a coaching style to provide technical and managerial guidance as a professional society that has a public responsibility. The SCGO took this opportunity to develop institutional capacity and leadership and attracted over 300 physicians across the country in 2018.

A complementary role and joint society initiatives could contribute to moving activities forward. Achieving a goal as a joint activity between professional societies is a somewhat novel approach. Their moral authority as national professional societies can lead to building both ownership and leadership. This approach could be a key factor in sustainability for developing a system for cervical cancer management.

Quality is essential for cancer care. The stepwise quality-focused approach taken in this project revealed opportunities and barriers in each step of the comprehensive cancer care continuum. Solutions to address the barriers were discussed and responded to on time, such as a low participation rate of screening to the modification of teaching materials and adoption of the call-recall system.

Since the call for action to end the suffering caused by cervical cancer by the Director General of WHO in May 2018, countries and partners are working on developing a global strategy towards the elimination of cervical cancer (*18*). A wide-scale awareness campaign to create knowledge as well as demand for screening is usually used for scaling up intervention. Before the potential demand is created, service availability for screening, diagnosis and treatment, *i.e.*, a capable workforce and functioning medical device, need to be in place. The small sample of intervention at the national level limits the reliability of application, especially with a capable workforce. However, in any level of service delivery, capacity building of service providers can be facilitated in a cascading manner by the SCGO under collaboration with the JSOG.

In conclusion, cooperation between SCGO and JSOG was the key for practice of the Cervical Cancer Prevention and Control Program in Cambodia. Contributing factors are based on joint society initiatives and a step-wise quality-focused approach. This effort might serve as a good example of how professional societies can contribute to capacity building and system development for prevention and control of cancer in LMICs.

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