BioScience Trends

**International Research and Cooperation Association
for Bio & Socio-Sciences Advancement**

Surveillance and vaccination
for polio eradication

In Lao PDR

BioScience Trends

BioScience Trends is an international journal published monthly by *International Research and Cooperation Association for Bio & Socio-Sciences Advancement* (IRCA-BSSA).

BioScience Trends publishes original research articles that are judged, after editorial review, to make a novel and important contribution to the understanding of any fields of BioScience including life science, clinical research, public health, medical care system, and social science. In addition to Original Articles, BioScience Trends also publishes Brief Reports, Reviews, and News & Letters to encourage cooperation and networking among researchers, doctors, and students.

Subject Coverage: Life science (including Biochemistry and Molecular biology), Clinical research, Public health, Medical care system, and Social science

CONTENTS   *Volume 1, Number 2, 2007*

CONTENTS   (*Continued*)

Guide for Authors

Copyright

An international effort to cure premature ageing

Richard Faragher[1], Makoto Goto[2]

Human civilizations are remarkably diverse. However, their citizens share a common cliché that the only certainties in life are death and taxes. Now, in the developed world there is a third virtual certainty; which is that death will come as a consequence of old age. This population ageing is the central demographic fact of the 21st century. By 2030 anywhere from 25% to 40% of first world citizens will be over 65. Living this long is a testament to medical progress.

Unfortunately, ageing is associated with an increased chance of death or the development of long-term morbidity. UK morbidity costs (in 1995) were estimated to be anywhere between £14 to £45 billion and are predicted to rise to somewhere in the region of £30 to £65 billion by the year 2030. These costs reflect a tremendous burden of human misery arising from poverty and poor quality of life. Improving healthy life for older people is thus the key challenge faced by biomedicine today.

Ageing is the unprogrammed consequences of mutations or processes favouring early life fecundity. It is innately complex and multifactorial rendering it a difficult area of research in which to apply post-genomic technologies. This has led to a need for more tractible model systems which recapitulate some, but not all aspects, of the normal ageing process. Perhaps the most famous of these is Werner's syndrome; an autosomal recessive genetic disorder in which

Figure 1. Meeting participants in front of Keio Plaza Hotel, Shinzyuku, Tokyo.

Table 1. Werner Syndrome Consortium Meeting Programme

Day 1

- Opening Remarks. *Makoto Goto, MD. Toin University of Yokohama, Japan*
- British ageing research: an overview of collaborative opportunities. *Richard Faragher, PhD. Brighton University, UK*
- Werner syndrome updated. *Makoto Goto, MD.*
- Clinical features of Werner syndrome: from a metabolic point of view. *Koutaro Yokote, MD. Chiba University, Japan*
- Stress, immunity and ageing. *Janet Lord, PhD. Birmingham University, UK*
- The role of stress kinases in the replicative lifespan of Werner's syndrome cells. *Terry Davis, PhD. University of Cardiff, UK*
- Homologous recombination and the Werner syndrome protein. *Kiyoshi Miyagawa, MD. University of Tokyo, Japan*

Day 2

- Chemical synthesis in the study of accelerated aging. *Mark Bagley, PhD. University of Cardiff, UK*
- Searching for hints of drug discovery from the study of Werner syndrome. *Yasuhiro Furuichi, PhD. GeneCare Institute, Japan*
- Biomarker discovery for Werner syndrome,using proteomic serum profiling. *Naohisa Tomosugi, MD. Kanazawa Medical University, Japan*
- Alteration of IgG oligosaccharide structure during normal and accelerated ageing. *Munehiro Nakata, PhD. Tokai University, Japan*
- What has replicative senescence got to do with organismal ageing? *Richard Faragher, PhD.*

Day 3

- Transcriptomic analysis of replicative senescence. *David Kipling, PhD. University of Cardiff, UK*
- Gene expression profiling in Werner syndrome family. *Yuichi Ishikawa, MD. Japan Cancer Institute, Japan*
- Modelling wrn function using Drosophila melanogaster. *Robert Saunders, PhD. The Open University, UK*
- Dermatological features and collagen metabolism in Werner syndrome. *Atsushi Hatamochi, MD. Dokkyo University, Japan*
- DNA microarray analysis of Werner and Werner-like syndrome. *Yasuhito Ishigaki, PhD. Osamu Nikaido, MD. Kanazawa Medical University, Japan*

individuals prematurely display many, but not all, of the features of normal human ageing. Although the identification of the mutation causing Werner's syndrome took place in America, this was underpinned by the outstanding work of the Japanese clinical community, which identified more than three quarters of all the world's Werner's syndrome patients. In the United Kingdom, research into the fundamental biology of the disease has provided a mechanistic understanding of how the disease operates at the cell and tissue level. These developments set the scene for the first meeting of the Anglo-Japanese Werner's syndrome consortium in Tokyo earlier this year. This consortium is funded by the Biotechnology & Biological Sciences Research Council of the United Kingdom with the goal of sharing resources and expertise. Space precludes a full description of the science presented however we have listed the subject areas covered in Table 1 so that readers can source key literature references independently.

The central scientific points of the meeting were (*i*) that existing drugs and improved care regimes now have the potential to considerably increase the life expectancy of Werner syndrome patients (*ii*) that the most likely cause of primary pathology in the disease is premature cellular senescence (with concomitant activation of p38 map kinase signaling); raising the possibility that inhibitors of this pathway currently in clinical trails may be realistic future therapies for the disease and (*iii*) that an intact Werner's syndrome helicase is a key molecular requirement for the development of many different types of cancer. On a more general note, the meeting showed that real advantages can be gained by providing research funding which allows chemists, biologists and clinicians to collaborate across national boundaries on a single biomedicial problem. Werner's syndrome research in particular is now shifting towards the type of multi-national, multi-disciplinary mode of research which inspired the Editors to found *Bioscience Trends*. It is hoped that we will have significant biomedical progress to report in the years to come.

([1]*SPARC Programme Director, School of Pharmacy & Biomolecular Science, University of Brighton, Brighton UK;* [2]*Division of Anti-Ageing & Longevity Sciences, Department of Clinical Engineering, Faculty of Medical Engineering, Toin University of Yokohama, Yokohama, Kanagawa, Japan.*)

Human cases of H5N1 avian influenza in indonesia: The need for international assistance

Kazuhiro Kakimoto[1, 2,]*, Ariestya Anggraeni[3]

Key Words: Avian influenza, Indonesia, human cases

Since 2003, 321 confirmed human cases of H5N1 avian influenza (AI) and 194 deaths worldwide have been reported to the WHO as of August 2007. More cases occurred in Indonesia than in any other country, accounting for approximately one-third of cases worldwide.

The Government of Indonesia responded to the epidemic by establishing the "Indonesia National Committee for Avian Influenza Control and Pandemic Influenza Preparedness," a ministerial-level committee, and developing the "National Strategic Plan for Avian Influenza Control and Preparedness for Human Pandemic Influenza 2006-2008." The government's most noted step has been the Avian Influenza Campaign, conducted in collaboration with the Government of Japan and UNICEF, using TV and radio public service announcements, free public concerts, education for journalists, print materials (Figure 1), and other media to promote simple, effective habits that can reduce the risk of contracting bird flu and detect suspected cases earlier. A surveillance system for human cases of AI is still being established by the Ministry of Health with programs targeting district health offices to train AI surveillance officers who are responsible for locating suspected cases at hospitals, health centers, and communities, reporting them to the government, and liaising with the community. In addition, the ministry appointed two national, eight regional, and 34 sub regional laboratories as reference laboratories and 100 hospitals as AI referral hospitals.

According to reports published by the Health Ministry, 105 confirmed cases and 84 fatalities have been reported since 2003, indicating a higher fatality rate than in other countries. A review of 28 cases, including 23 deaths, out of 30 human cases of AI, including 26 fatalities, with adequate information that were reported from January 2007 to August 2007 indicated that 10 cases (35.7%) lacked any clear contact to dead or sick birds; of the 23 fatalities, 15 cases (65.2%) were diagnosed postmortem as H5N1 with RT-PCR. Of note is that some cases involved no clear contact to dead or sick birds and more important is the fact that the majority of the cases were diagnosed

Figure 1. IEC material used in Indonesia with four key messages printed on the fingers: 1) "Don't touch sick or dead poultry," 2) "Wash your hands and cooking accessories before use," 3) "Keep poultry separate from people" and 4) "If you notice influenza-like symptoms and fever after coming in contact with poultry, visit a health center."

as H5N1 postmortem. Since a delay in detection, diagnosis, and treatment leads to a delay in the chance to contain probable pandemic influenza, these findings suggest that challenges remain in the form of establishing early case detection in humans and mechanisms of early diagnosis.

In Indonesia, the surveillance system for humans as is integrated with the surveillance system for animals and laboratory networks must be enhanced along with capacity building. Additionally, Indonesia's financial and technical needs are a global issue that the international community needs to be better informed about. Although some believe that the threat of AI and pandemic influenza will pass in the near future, the belief that the H5N1 virus cannot be transmitted to humans is no longer valid and human experience with pandemics such as Spanish influenza should not be forgotten.

([1]*International Medical Center of Japan, Japan;* [2]*Ministry of Health (JICA advisor), Indonesia;* [3]*Ministry of Health (JICA advisor office), Indonesia.*)

Risk of radiation exposure from genbaku and genpatsu: The 1945 atomic bombings and the 2007 Kashiwazaki nuclear power plant leak

Chushi Kuroiwa*

The Department of Health Policy and Planning, School of International Health, Graduate School of Medicine,
The University of Tokyo, Tokyo, Japan.

Key Words: Atomic bomb, radiation disease, nuclear power plant, Kashiwazaki, Chernobyl

The Japanese word for atomic bomb is "genbaku" while that for nuclear power plant is "genpatsu". The two words only differ by one character, but both refer to the same radioactive substances - uranium and plutonium - and both have the potential to bring about catastrophe for innocent people (*1*). Terrorists could make atomic bombs, or genbaku, by stealing uranium and plutonium from nuclear plants, or genpatsu.

This year, Hiroshima and Nagasaki marked the 62nd anniversary of the world's first atomic bombings; the attack by the American B-29 bomber Enola Gay on August 6 of 1945 killed 140,000 in Hiroshima instantly or within a few months (*2*), and another B-29-dropped plutonium bomb on August 9 killed approximately 74,000 people in Nagasaki (*3*).

As in past decades, the approach of the anniversaries of the bombings has been marked by media sources throughout Japan reporting hidden stories behind the bombings. One such report drew my interest as a Japanese pediatrician. Mainichi newspaper reported on August 4 that at Omura Naval Hospital, located 19 kilometers away from the hypocenter in Nagasaki, medical personnel who treated patients suffering from the August 9 atomic bomb were found to develop higher rates of radiation diseases such as cancer, liver ailments, or cataracts (*4*). Thousands of atomic bomb survivors were transferred to the hospital by relief train or truck, and some 860 medical staff members worked to save the victims. A questionnaire survey was conducted from November 2005 to November 2006 by lawyers representing some 267 atomic bomb survivors who have not yet been recognized as radiation disease sufferers. Under the current law, those who did not live within 2 kilometers of the hypocenter on the date of the bombing are not classified as radiation disease sufferers. The lawyers pressed the government to recognize the victims as radiation disease sufferers, making them eligible for special medical allowances. Of the 73 medical personnel working at Omura Naval Hospital on the day the bomb was dropped in 1945, 25 (34.2%) developed cancer, a figure significantly higher than the

9.7% observed in the control group. Prof. Shoji Sawada of Nagoya University, one of the survey members, said that the hospital workers could have inhaled radiation particles attached to the clothing or hair of the atomic bomb survivors, causing continuous radiation exposure from within their bodies (*4*).

"I am not exaggerating, but even without using a stethoscope, you could hear the glass shards jangling against each other every time they took a breath. There were many patients like that. ….So many people came at once that we didn't use a stethoscope for every one of them, we just put our ears to their chests and listened to determine if their hearts were still beating. …. At first, we didn't understand radiation sickness, and we didn't start to notice anything unusual until three or four days later. For example, when we gave a patient an injection, the skin in the area of the injection would gradually decay, and leukemia would eventually set in. When the girls in the volunteer corps borrowed combs from the nurses and used them, their hair would fall out from the roots. We thought this was strange. There was the shock and sadness of young women who suddenly lost all their hair," said Dr. Masao Shiotsuki (1920-1978), who treated the victims of the atomic bombing at Omura Naval Hospital in 1945 (*5*). One can easily imagine how medical staff might have come into close contact with the victims and how powerful the tiny radiation particles must have been.

Japanese people are all too familiar with the issue of radiation exposure; the government of Japan, however, has given priority to the general construction business, which includes the construction of nuclear power plants and may expose the residents near the plants to radiation. As the BBC frankly reported, "It seems odd, then, that it is so addicted to nuclear energy, operating more reactors than any other country after the United State and France" (*6*). This report came after a strong earthquake hit northwestern Japan on July 16 of this year, causing a fire and damaging the world's largest nuclear power plant in Kashiwazaki, Niigata Prefecture. The plant was ordered to close indefinitely on July 18

amid growing public anger over revelations that damage was much worse than initially announced. The damage included the spilling of radioactive water and waste into the sea and atmosphere (*7*). Concerns about nuclear safety have echoed across Japan, which depends on 55 reactors for about 30 percent of its electricity needs.

Although officials repeatedly announced that the extent of the radiation leaks was extremely small and not at a level that would pose health hazards, hotels and inns in Kashiwazaki have received roughly 30,000 cancellations of room reservations as of August 4. This indicates that majority of Japanese citizens may have doubts about the government's announcements proclaiming safe levels of radiation leaks, and residents throughout the prefecture have pressured the governor into persuading the central government to allow an inspection team of the International Atomic Energy Agency (IAEA) access to the facility. The government, initially reluctant to let the IAEA inspect, relented and the IAEA team started investigating the damaged plant on August 6 (*8*). The residents hope an announcement by the IAEA will be sufficient to convince people across Japan of the lack of danger associated with the extent of the leaks and encourage them to come to the resorts for summer vacation, as in previous years.

A peaceful population living under a pacifist constitution (*9*), the Japanese have finally grasped that the government's top priority is the interests of its own groups rather than the lives of we, its citizens. It consistently attempts to hide realities behind vague remarks. The then Prime Minister Shinzo Abe has not answered recent questions concerning issues such as a series of scandals involving various Ministers, including one culminating in the suicide of the agriculture minister and another relating to false pension records kept by the Social Insurance Agency. This attitude on the part of the government has not changed for more than 60 years. Dr Shiotsuki recalled the date in 1945 when he treated the victims sent from Ground Zero of the atomic bombing: "When the first truck arrived-and I still can't forget this-but I thought, well, now the War is over. Once they understood the real situation, the Japanese military, no matter how single-mindedly they wanted to continue fighting, would stop once they knew the facts. Anyone in the military who still wanted to continue fighting against a weapon that had caused this much harm to people had to be crazy. I thought once any normal person had seen this, they would realize they should end the war" (*5*).

Mr. Kyuma was forced to step down as defense minister in early July after commenting in a speech, "I understood the bombing brought the war to its end. I think it was something that couldn't be helped." The remarks outraged atomic bomb survivors and others who interpreted them as a suggestion that the atomic bombings were justified (*10*). However, the situation seems as if the government has evaded its responsibility

in the war by simply sacking the naive defense minister. From the theoretical point of view of the civilians, his remarks may indicate that the rightwing Japanese government of that time could have saved more than 200,000 innocent civilians if Japan had only surrendered earlier. The Japanese population was also a victim of the government at the time, although the atomic bombs can certainly not be justified and there must have been some other way to end the war.

The accident at the Chernobyl nuclear power plant in 1986 was the most severe in the history of the nuclear power industry, causing a massive release of radiation over large areas of Belarus, Ukraine, and the Russian Federation. The Chernobyl Forum held an international conference entitled "Chernobyl - Looking Back to Go Forward" in Vienna from the 6th to the 7th of September, 2005 (*11*). In the report, 28 persons were reported to have died in 1986 due to causes directly attributable to acute radiation syndrome. Although the reliability of cancer mortality cannot be accurately assessed, the international group of experts also predicted that, among the 600,000 persons receiving more extensive exposure, the possible increase in cancer mortality due to this radiation might be as much as a few percentage points. Finally, more than 4,000 children were reported to have developed thyroid cancer (*12*). In addition, elevated frequency of chromosome aberrations in most residents tested was reported (*13*), and the frequency of congenital malformation in newborns and human embryos has increased in heavily contaminated areas of Belarus following the accident (*14,15*). Yuri E. Dubrova *et al*. reported in 1996 that the frequency of germline mutations was found to be two times higher in the exposed families than in the control group (*16*), and a 2002 report by the team suggested that the elevated minisatellite mutation rate can be attributed to post-Chernobyl radioactive exposure (*17*).

The Liberal Democratic Party of Japan (LDP), the ruling party of Japan, was devastatingly defeated in the Upper House elections on July 29 of this year, and the massive defeat will greatly hinder efforts by the LDP to pass bills in the Diet. For the first time, Japanese voters clearly expressed their dissatisfaction with the current regime (*18*). This is a chance for the public to discuss, free of pressure from a government held captive by scores of vested interests, the potential for an eco-friendly society in Japan, whether to stop the operation of all nuclear plants, and the introduction of new transport and urban planning measures concerning the environment. Even foreigners are sure to question why nuclear plants are located near resort areas, as in the case of the Kashiwazaki plant, and why the Hamaoka nuclear plant is situated in the Tokai area, where a magnitude-8-class earthquake of incredibly massive proportions is predicted. The situation simply does not make sense.

Japan has started preparations for the G8 summit in

Hokkaido next year, and the main item on the agenda is the Environment (*19*). Without solving these serious domestic problems associated with the existence of nuclear plants in Japan, what sort of paramount message can Japan convey to the world?

(**e-mail: ckuroiw@m.u-tokyo.ac.jp*)

References

1. Nishio B. Genpatsu and Genbaku, only one different letter. In 50 stories thinking risk of nuclear plants. 2006. Iwanami Shinsho,Tokyo.
2. 'Hiroshima was a hell': A-bomb day hears Abe nonnuclear vow. The Japan Times. Aug 7, 2007.
3. Johnston E. Nagasaki mayor slams nuclear talk, tests. The Japan Times. Aug 10, 2007.
4. Nagasaki Genbaku: Relief-Hibakusha caused lots of cancers. Evidence for recognition. Mainichi newspaper. Aug 4, 2007 (Japanese). http://www.mainichi-msn.co.jp/science/news/20070804k0000m040146000c.html (accessed Aug 11, 2007).
5. Dr. Masao Shiotsuki. Testimonies of the Atomic bomb survivor. In Nagasaki and peace. http://www2.nbc-nagasaki.co.jp/peace/voices/en-no16.html (accessed Aug 10, 2007).
6. Japanese fears over nuclear power. BBC world news. July 17, 2007. 17:09 GMT.
7. Japan admits more nuclear leaks. BBC world news. July 17, 2007. 14:35 GMT.
8. IAEA begins assessing quake-hit N-plant. The Japan Times. Aug 7, 2007.
9. Rabindranath Tagore and Japan. Editorial. The Japan Times. Sep 7, 2003.
10. Abe pledges to review rules for A-bomb health support. The Japan Times. Aug 6, 2007.
11. Chernobyl - Looking Back to Go Forwards 2005. http://www-ns.iaea.org/meetings/rw-summaries/chernobyl-conference-2005.htm (accessed Aug 10, 2007).
12. IAEA, WHO, UNDP, FAO, UNFPA, UN-OCHA, UNSCEAR, World Bank Group, Belarus, the Russian Federation, Ukraine. Recommendations to the Government of Belarus, the Russian Federation and Ukraine. The Chernobyl Forum: 2003-2005. http://www.iaea.org/Publications/Booklets/Chernobyl/chernobyl.pdf (accessed Aug 10, 2007).
13. Bochkov NP. Analytic review of cytogenetic studies after the Chernobyl accident. Vestn Ross Akad Med Nank 1993;6:51-56.
14. Lazjuk GI, Kirillova IA, Nikolaev DL, Novikova IV. In: The Chernobyl Papers Vol. 1. Doses to the Soviet Population and Early Health Effects Studies (Mervin SE, Balanov MI, eds.). Research Enterprises, Richland, WA, USA, 1993; pp. 385-397.
15. Lazjuk GI, Kirillova IA, Dubrova YE, Novikova IV. Incidence of developmental defects in human embryos in the territory of Byelarus after the accident at the Chernobyl nuclear power station. Genetika 1994;30:1268-1273.
16. Dubrova YE, Nesterov VN, Krouchinsky NG, Ostapenko VA, Neumann R, Neil DL, Jeffreys AJ. Human minisatellite mutation rate after the Chernobyl accident. Nature 1996;380:683-689.
17. Dubrova YE, Grant G, Chumak AA, Stezhka VA, Karakasian AN. Elevated minisatellite mutation rate in the post-Chernobyl families from Ukraine. Am J Genet 2002;71:801-809.
18. A devastating defeat for Mr. Abe. Editorial. The Japan Times. July 31, 2007.
19. The government instructs environmental issue as agenda for 2008 G8 summit in Japan. July 5, 2007. Mainichi Newspaper (Japanese).

Sociodemographic characteristics, sexual behavior, and HIV risks of rural-to-urban migrants in China

Na He*

Department of Epidemiology, School of Public Health, Fudan University, and The Key Laboratory for Public Health Safety of Ministry of Education, Shanghai, China.

SUMMARY	Because of China's increasing industrialization over the past two decades, many rural residents have migrated to urban areas but frequently return to visit their home villages. These rural-to-urban migrants are generally young, are better educated than non-migrating rural residents but less educated than their urban counterparts, are most likely employed in venues such as construction sites, factories, and commercial services, are usually separated from their families, and have limited access to health services. Risky sexual behaviors such as premarital sex, extramarital sex, and commercial sex are increasingly observed among rural-to-urban migrants. Some female and male migrants are themselves working as sex workers in urban areas. Although the rates of HIV infection and other sexually-transmitted infections (STIs) are still relatively low in general rural-to-urban migrants, they are rising in migrant sex workers. Increasingly observed risky sexual behaviors and prevalent STIs among migrants strongly suggest the serious potential for an expanding HIV epidemic in and beyond these migrant populations. Rural-to-urban migrants play a critical role in the spread of HIV in China and are an important target for HIV prevention and intervention programs. Such programs should be socially, demographically, and culturally tailored to rural-to-urban migrants.

Key Words: Sexual behaviors, HIV, sexually transmitted infections, rural-to-urban migrants, sociodemographic characteristics

Introduction

Since the report of the first AIDS case in 1985, the epidemic of HIV/AIDS in China has gone through an initial sporadic period, followed by a localized epidemic, and has now reached a stage of rapid growth. No province in China has been HIV-free. The HIV epidemic in China is now spreading from the originally infected groups, injection drug users (IDUs) (mostly minorities) and blood/plasma donors (mostly farmers), to sexually active heterosexuals. Patients infected through sexual transmission are now the fastest growing group, accounting for close to 50% of new infections in 2005. Overall, they represent 43.6% of total HIV/AIDS cases, including commercial sex workers or their clients (19.6%), partners of HIV-infected individuals (16.7%),

and men who have sex with men (7.3%) (*1*). In the meantime, experts estimate that more than eight million Chinese citizens acquired sexually transmitted diseases (STDs) in 2002, and this figure is growing annually by almost 40%, which is far greater than the official figure of 830,000 STD cases in China in 2002, with an estimated annual rate of increase of 20-30% (*2*). Given the fact that STD patients are more susceptible to HIV infection and are more infectious once infected with HIV, the hidden pandemic of STDs in China further suggests the potential explosion of HIV infection through the mode of sexual transmission.

The number of reported HIV cases in China totaled about 203,527 by April 30, 2007 (*3*), but the official estimate of people living with HIV was 650,000 (*1*). The majority of these individuals were rural residents and not identified (*1,4*). If there had been no population migration, the HIV epidemic might have been and continue to be localized in rural areas in China. However, the China National Bureau of Statistics estimated that in 2000 there were 121 million migrants in China, 73% of them from rural villages (*5*). Such

*Correspondence to: Department of Epidemiology, School of Public Health, Fudan University, and The Key Laboratory for Public Health Safety of Ministry of Education, Shanghai 200032, China;
e-mail: nhe@shmu.edu.cn
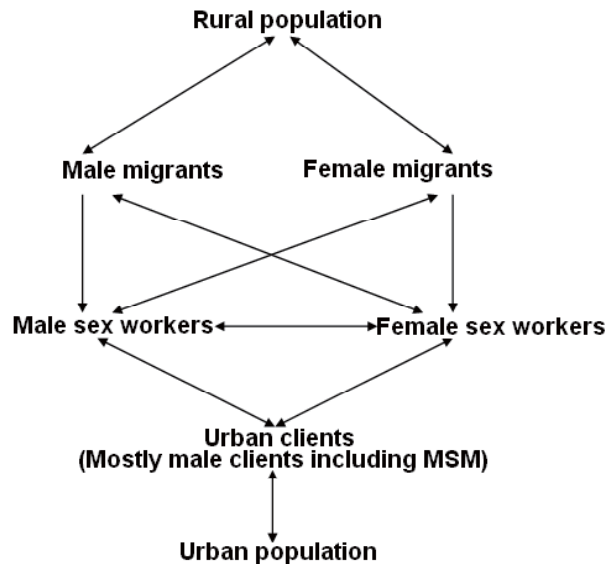
Figure 1. Potential HIV transmission between rural and urban populations in China.

a mass internal rural-to-urban migration might shift the HIV epidemic as a result of broadening social and sexual mixing (*6-13*).

A great potential for HIV transmission between rural and urban populations and most likely from rural to urban populations is suspected in the current stage of the disease's spread, as rural-to-urban migrants may play a key role in bridging the epidemic between these two populations (Figure 1). Some male rural migrants may buy commercial sex from female sex workers (FSW) in urban areas who are also approached by male urban residents. If clients of either type are HIV-infected, then the virus can be transmitted from one type to the other. Consequently, HIV-infected male clients from both rural migrants and urban residents may transmit the virus to their wives, girlfriends, and other sex partners through unprotected sexual behaviors. Furthermore, if a promiscuous sexual pattern exists among rural and/or urban residents, an HIV epidemic is inevitable in the general rural and/or urban populations. In the mean time, some male rural migrants are themselves commercial sex workers, including those predominantly providing commercial sex to men who have sex with men (MSM) in urban areas. This may open another path for HIV transmission between rural and urban populations (Figure 1). Transmission of HIV and STDs by migrants is one of the most serious public health challenges that China must face and is the most difficult to control.

Careful examination of demographic characteristics, social networks, psychosocial characteristics, and economic status of rural-to-urban migrants as well as their sexual behavioral patterns and trends is needed in order to better understand the role of rural-to-urban migrants in the spread of the HIV epidemic in China. Such knowledge is essential not only to improve the health of migrants but also to protect the public

health of both urban and rural populations. In this regard, this article reviews existing literature on social, economic, and demographic characteristics of rural-to-urban migrants and follows it with existing evidence linking sexual behaviors and HIV/STI risks among and beyond migrant populations. Last, implications of these findings for future social epidemiological research on HIV/AIDS and the design of effective HIV/AIDS interventions are discussed.

Rural-to-urban migration: a fast-growing socially controversial phenomenon

When the Chinese Communist Party took over mainland China in 1949, it imposed external and internal travel restrictions. It established a household or permanent residency registration system (known as "hukou" in Chinese) throughout the country, under which everyone is assigned to a particular place of residence and the Chinese population is officially divided into urban and rural residents. Individuals who wish to move to another part of the country must receive approval from the appropriate authorities to establish another "hukou". The extremely strict permanent residency registration system was closely related to people's social security benefits, residence, education, and working and living conditions. Accordingly, migration was not a common phenomenon until the early 1980s in China. The policy of "Reform and Opening-up" implemented in the early 1980s is what allowed people to move freely and opened urban areas to peasants from rural areas. Although the permanent residency registration system is still valid, it is no longer as closely related to working and living situations as it had been from the 1950s to the 1970s. Therefore, large numbers of people have migrated between different urban areas, and particularly between urban and rural areas, for various economically related activities. The number of migrants has been increasing dramatically during the last two decades, reaching more than 50 million in 1995 and more than 120 million in recent years. The majority of migrants are peasants who left their rural home villages in search of better economic prospects in economically developed cities such as Shanghai (the adopted home of an estimated 5 million migrants).

The migration of large numbers of rural people to urban areas has been controversial and vigorously debated in terms of benefits to the social and economic development of China. On the one hand, the migration has considerably accelerated urbanization of China and made tremendous contributions to social and economic development of both source and destination areas. Many migrants are now working in construction, community services, and commercial services in cities, which are essential to the life and development of cities, but work in these fields is usually laborious, dirty, and low-paid, so permanent city residents are

very reluctant to take such jobs. In spite of low wages, migrants still make a great deal more money than they would had they remained farmers at home and they can save money. Studies have indicated that rural migrants send back large amounts of money to their hometowns every year, which can be a substantial part of the annual finances of local communities and dramatically promote economic development of these rural communities (*14,15*). By living and working in urban areas, rural migrants may be educated in modern technologies and cultures. When they return to rural areas, these new techniques and modern cultures are also brought to rural areas, dramatically promoting the social and cultural development of such areas (*16*). On the other hand, the huge numbers of rural migrants in cities has caused many social problems and placed stress upon permanent city residents. The migrants now compete with permanent city residents for limited resources such as certain jobs, public transportation, utilities, housing, and public health services (*16-18*). Some migrants may be criminals on the run or may become criminals due to unemployment and are consequently regarded as a primary source of urban violence and crime (*19-21*).

Sociodemographic characteristics and social networks

The main reason that rural people leave their home villages or towns for large cities is the huge gap in economic development between rural and urban areas in China (*15,22,23*). However, not every rural migrant is accepted by urban society; *i.e.*, urban society selects certain migrants. On the other hand, most migrants carefully select their destination cities based on available information from various sources. Thus, migration is actually the result of two-dimensional selection. Keeping this in mind, some common characteristics of migrants may be discerned. First, from the perspective of source areas, only relatively young and well-educated people have more sources of information about job opportunities, more motivation to look for a better life, greater capacity to work and live alone in a different location, and are thus more likely to profit from their migration. Second, from the perspective of destination areas, young people with better education are more welcome and are thus more likely to be employed in cities.

Age distribution

As noted above, the majority of migrants are young adults. According to a nationwide 1% population sampling survey in 1995, 86% of migrants were between 15 and 65 years of age, and 71% were between 15 and 34 years. Young people in the age group of 20-24 years accounted for 31% of the entire migrant population (*24*). In Shanghai in 1993, more than half (53.7%) of migrants were 20 to 34 years old. This

proportion was higher in 1997, when it reached 55.4% and was even higher in 1999 (*25,26*).

Education

Although rural migrants are less educated than their city counterparts, they are relatively better educated than those staying in their home villages and tend to have a higher education level than the overall average nationwide. Based on the same nationwide 1% population sampling survey in 1995 (*25*), 17% of migrants were educated to high school or college level, while only 11% reach this level among rural populations. Forty-one percent (41%) of migrants had been educated in secondary schools, while only 31% attained this level in rural populations. However, there was still a large proportion of migrants (42%) who were illiterate or had primary schooling only, although this proportion was significantly lower than that of the entire rural population (58%). In an study of migrants in Shanghai, 12% of migrants were educated to high school or college level, 48% to secondary school level, and 28% to primary school level, with the remaining 9.1% of migrants being illiterate or semi-illiterate (*27*).

Gender distribution

A deep-rooted cultural belief in rural areas of China is that females are usually housekeepers, so they typically receive less education than males and are expected to stay at home. This is reflected in the disproportionate amount of migration between males and females. The gender ratio among rural migrants was 258 males to 100 females, while the overall gender ratio is 108 to 100 in rural areas (*24*). A study in Beijing revealed that as many as 77% of migrants were males (*22*). This was also true in Shanghai, where 62.9% of migrants were males in 1997 and 65.5% in 1999 (*24,25*). However, in certain working venues, such as some clothing and toy factories and some electronics companies, female migrants are usually more numerous than males. For example, about 78% of the workers in processing factories in an economically developed city (Dongguan) of Guangdong province were females (*28*).

Marital status

Previously, there was general agreement that there are more married than single males but more single females than married females among the migratory population. There were two main reasons for this phenomenon. One was that once married, females would usually stay at home in the countryside to give birth or to take care of the family unless a woman's husband needed her help, which most likely occurs when the couple operates a small business or a grocery shop in the city; on the other hand, husbands are expected to work hard outside

the home and save money for the family. The second reason is that some private enterprises or industries exclude or do not welcome married females for employment. According to estimates of the government of Guangdong province, about 45% of female migrant workers were aged 15 to 19 years in 1990 in Dong-guan, a city near the capital of the province. These young females were most likely single (*28*).

These observations may be no longer relevant now. A study on migrant workers in Shanghai indicated that nearly 40% of migrant workers are single, and this proportion was the same for males and females (*26*). Observations indicate a rapid rise in the proportion of married individuals among migrants in recent years.

Residency period

Migrants now tend to reside longer in their destination cities than ever before. In Shanghai, the proportion of migrants who have lived in Shanghai for more than six months increased from 49% in 1993 to 70.1% in 1997. More than 36% of migrants had resided for more than one year. In addition, about 46% of migrants in Shanghai hoped to work and live there permanently (*26*). In Beijing, about 54% of migrants had resided for more than six months, and 23% had resided for more than a year. Nearly 50% of migrants expressed a strong willingness to permanently reside in Beijing (*22*). In a sampling survey in Beijing in 1999, 69.4% of migrants had lived in Beijing for more than six months (*29*). A similar trend has also been observed in Guangzhou, the capital city of Guangdong province in southern China, which is a major destination city for rural migrants (*30*).

Occupation

The number of migrants who have found employment has been increasing along with the increasing number of migrants arriving in the cities and the proportion of migrants who are employed in cities. These migrants predominantly work in agriculture and receive low wages. However, there is a clear trend toward more and more migrants entering occupations with higher wages (*23*), such as in construction, factories, various commercial and community services, house cleaning and babysitting, and self-employment (*22,24,31,32*). Most of these jobs are so laborious, low-paid, demanding, and/or dirty that city residents avoid such employment. Because of this, migrants are discriminated against by city residents in most settings. Some female migrants have entered into commercial sex services for various reasons, further accelerating such stigmatization (*15*). In certain cities, the occupation of a migrant is associated with the province that he came from. For example, in Beijing, migrants from Hebei province usually work at construction sites or build houses, migrants from Zhejiang province

usually work in markets or are self-employed in other commercial services, migrants from Henan province usually recycle trash, and migrants from Anhui province usually work as house cleaners, babysitters, or provide community services (*24*).

Living conditions

Rural migrants usually live in areas geographically separate from the communities of city residents. Rural migrants tend to live amongst people who come from the same province or prefecture in a place or community that is most likely located between suburban and urban areas around a city. They may live in dormitories provided by employers or in rented apartments. The living conditions are usually very poor, with extremely limited bathing or shower facilities. There are only a limited number of public lavatories, which are seldom cleaned. There is very little privacy in such living conditions because migrants usually share a dormitory room or an apartment with other migrants. Security is also problematic, so many migrants keep their identification cards or certain certificates and money in their pockets. They usually only keep a small amount of money and send the rest back home. Most recently, clusters of these migrants, such as "Anhui village", "Henan village", "Xinjiang village", "Zhejiang village", and "Wenzhou (a prefecture of Zhejiang province) village", have sprung up in Beijing, Shanghai, and Guangzhou.

Most likely, migrants live in a joint location or community connecting urban and suburban areas; *i.e.*, in the marginal areas of a city (*33-36*). By doing so, they are actually establishing relatively isolated communities of their own, which are less likely to be integrated into the communities of city residents. Migrants are thus psychologically resistant to city residents, and city residents tend to regard migrants as intruders or foreigners, which makes migrants feel marginalized and depressed (*37,38*). Because they work very hard and yet have inferior living conditions, they feel a strong sense of injustice, and migrants have a very tenuous relationship with city communities and residents (*38*).

Entertainment and recreation

A systematic study or report on the entertainment or recreation of rural migrants in cities has yet to be conducted, but the authors' experience and observations indicate that these aspects depend on the social network of migrants. They may just stay in their apartments and watch TV or play poker, they may visit friends, coworkers, or relatives, or they may go to the movies or visit other public places of entertainment that are particularly appropriate to rural migrants in terms of cost and location. Although less frequent, there are

some young migrants who attend certain training workshops that are potentially useful to them in terms of finding better jobs or improving their lives.

Social networks

The process of migration of peasants is actually a required process of establishing new social relationships or networks to better acclimate to a new environment. Research has demonstrated that the sooner and the stronger a new social network in the city is established, the sooner and better the migrant is assimilated into city life.

Studies on the networking of migrants reveal that homogeneity is an important basis for the establishment of strong ties. Most rural migrants seek other rural migrants as friends in their social networking; only a few migrants seek local city residents as friends or partners. When confronted with difficulties in daily life or work situations, a migrant would most likely ask for help from family members, friends, relatives, and other migrants from their own village. Although less likely, they may seek help from coworkers, employers, policemen, or other local government officials, and possibly, their landlords. The intensity of their relationships is characterized by the frequency of their interactions, which are also an important indicator of trust, with trust being at the core of a migrant's social network. There are two different types of trust in a migrant's network, non-institutional and institutional. Relationships with family members, friends, relatives, peer partners, and coworkers are non-institutional, that is, they are solely based on blood and geographic ties. In contrast, policemen and other local government officials are powerful people in the community and are in a position to solve certain problems in a migrant's life. It is this power that forces migrants to communicate and further establish relationships with them, which are forms of institutional trust. However, non-institutional trust is the most important basis for establishment of intensive relationships. The stronger the intensity of relationships among migrant workers, the more likely their behavior is to be influenced. Some have argued that the primary social relationship is the main component of a migrant's social support network and that such social relationships function more effectively than social organizations in terms of changing social behaviors (*39*).

Access to health services

One major problem that migrants face is extremely limited access to health services due to limited information about health education and disease prevention available to them, low awareness of health promotion, and low income to pay for relatively expensive health services. In addition, they may encounter linguistic and psychosocial barriers to health services. Most migrants do not have health insurance. Therefore, when they are ill, they either choose to stay at home and treat themselves or go to an unqualified private clinic most frequently operated by another migrant who is probably poorly trained. If the illness is severe, they would most likely go back to their own hometowns or villages, where they could receive much less expensive treatment.

This can be particularly problematic for female migrants because many are of child-bearing age but lack access to the family planning system. A study conducted in Shanghai in 1999 indicated that the number of reported abortions among female migrants was 56,400, which was 5,400 more than that in 1998, and accounted for 26.17% of the total number of abortions. Notably, among the 56,400 cases, 29,300 were for married women and 27,100 were for unmarried women (*26*). The actual situation might be even worse, clearly demonstrating the importance and urgency of improving public health among migrants, and particularly females.

Because of low income or social security benefits, migratory status, and particularly because of low awareness of the importance of having children vaccinated, as well as a lack of knowledge of health facilities providing vaccination services, receipt of vaccinations among migrant children is generally lower than that among local city children. For example, the overall vaccination rate in the city of Suzhou, a wealthy city of Jiangsu province, which is very close to Shanghai, of four basic vaccines (bacille Calmette Guerin (BCG), oral live poliovirus vaccine (OPV), diphtheria-pertussis-tetanus (DPT), and measles vaccine (MV)) in 1999 was only 33.93% among migrant children aged 0-6 years, compared with an overall rate of more than 99% among local city children (*40*). In the city of Zhengzhou, the capital of Henan province, the vaccination rate among migrant children was reported to be 55.18% for BCG, 52.2% for OPV, 52.38% for DPT, and 48.93% for MV, with an overall rate for these four vaccines of only 32.13%, significantly lower than the overall vaccination rate of 99.1% among city children (*41*). In certain other cities, the situation has improved due to the tremendous efforts of health workers in those cities. For example, in the city of Guiyang, the capital of Guizhou province, the vaccination rate for all four basic vaccines was over 86% among migrant children. After active outreach to migrants, the vaccination rate for MV was even as high as 95.42% (*42*). In Beijing, a study indicated that the vaccination rates among migrant children were 77.8% for BCG, 83.2% for OPV, 82.7% for DPT, and 81.6% for MV (*43*). In Guangzhou, the capital of Guangdong province and the city with largest number of migrants, the vaccination rate for each of the four basic vaccines was over 90% among migrant children (*44*). Although

the vaccination rates among migrant children are much higher in these cities than in others, they are still relatively low compared to those among city children. Thus, much attention has to be paid to the improvement of vaccination rates among migrant children, as well as to the general public health of migrants.

Sexual behavior and HIV risks

In HIV research, there is a consensus with a growing body of work documenting the effects of migration and health (*7,8,45,46*). China's rural-to-urban migrant population has been repeatedly characterized as the tipping point for the HIV epidemic in China by the Chinese government and international societies (*1,46*). The difficulties of conducting HIV surveillance in this extremely volatile and marginalized population have been documented (*47*). Multi-city HIV surveillance data between 1995 and 2000 show that over two-thirds of the HIV cases involved rural-to-urban migrants. Eighty-five-point four percent of Beijing's and 74.4% of Shanghai's new HIV infections were found among migrants in the year 2000 (*47*).

Sexual behavior and HIV risks among typical migrants

Most rural-to-urban migrants are at an age where they are sexually active. HIV risk-taking behaviors such as premarital sex, commercial sex, and multiple sex partnership have often been observed in this population, and condoms are rarely used in these sexual acts. Condoms are even less likely to be used in their sex with spouses or other long-term sex partners. A cross-sectional study among 986 sexually active male rural migrants in Shanghai, China found that the proportion of men who had premarital sex had dramatically increased by year of birth (unpublished data, see Figure 2). In an exploratory study among a convenient sample of migrants including in-transit individuals in Beijing, and peddlers, restaurant workers, and job seekers in Shanghai, Anderson et al. observed that 24% (115/442) of the participants had had sex outside of a monogamous relationship (*46*). A study of 1,086 typical male migrants in Shanghai indicated that almost 80% of migrants reported that they had never used condoms, and among those who used condoms consistent use was also rare (*6*). About 11.5% of migrants reported having had sexual intercourse with commercial sex workers and among them, 75% never used condoms and only 16% reported consistent condom use (*6*). Data from 2,153 sexually active rural migrants in Beijing and Nanjing showed similar sexual risk behavior patterns and indicated a higher level of sexual risk among the Chinese migrant population in comparison to the non-migratory population (*48*).

However, the picture of HIV/STI infection rates among typical migrants is not as clear-cut. Using a
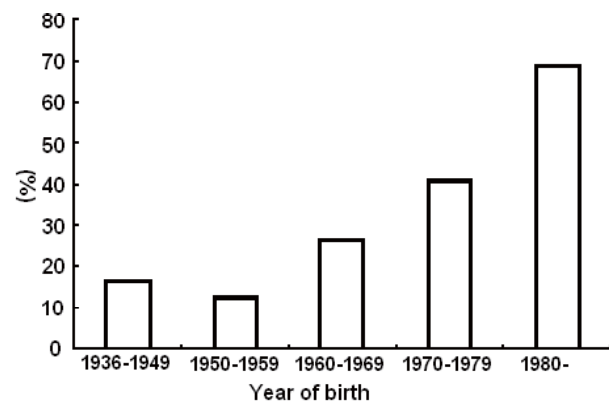
Figure 2. Proportions of having premarital sex by year of birth among male rural migrants in Shanghai, China.

dry-blood-spot technique in a cross-sectional survey among 4,148 migrants and 2,197 urban workers in Hangzhou, the capital city of Zhejiang province in eastern China, Hesketh *et al*. found a prevalence of syphilis of 0.68% among urban workers and 0.48% among migrant workers (*49*). A urine-based technique in a community-based cross-sectional study of 1,273 male rural migrants in Shanghai found a prevalence of chlamydia, gonorrhea, and syphilis of 3.5%, 0.5%, and 1.0%, respectively (*7*). Although these rates are low, there were sub-group differences. The prevalence of STDs was 3.2% for construction workers, 5.6% for vendors, and 5.6% for factory workers. Risk factors for STDs were: longer duration in Shanghai, frequent hometown visits, having multiple sex partners, and the desire to have multiple sexual partners. In a study of 1,536 randomly selected market stall vendors (all were rural migrants) in the city of Fuzhou in Eastern China, Detels, Wu, Rotheram *et al*. reported that the prevalence of any STD (based on testing) was 20.1% among those reporting sexual intercourse and 5.5% among those reporting never having had sexual intercourse (*50*). Among those reporting sexual intercourse, chlamydia was most common (9.4%), followed by herpes simplex II (9.3%). A total of 4.5% among those reporting never having sexual intercourse had herpes infection, but none had chlamydia. Recently, a cross-sectional study among 550 long-distance truck drivers in Tongling city of Anhui Province found about 17.4% of truck drivers had at least one STI: the prevalence of gonorrhea was 8.1%, that of syphilis was 0.7%, and that of herpes simplex II was 4.4% (*51*).

In sum, the limited set of studies seems to reveal that when using some biological measures for HIV/STIs, the prevalence rates tend to be lower among typical migrants when compared to high-risk populations such as female sex workers. However, studies using self-reported measures tend to have variable HIV/STI prevalence rates among typical migrants. An important point to keep in mind is that a majority of these studies did not use a population-based or sophisticated design (other than a convenience sample) to increase the

generalizability of their findings.

Sexual behavior and HIV risks among female sex workers

Some female rural migrants are working as sex workers in urban areas. They have limited knowledge about HIV/AIDS and do not consistently use condoms in sexual intercourse with both clients and long-term sex partners. Thus, female sex workers are generally at high risk of HIV infection. A cross-sectional survey conducted in Beijing and Nanjing reveals that for 666 sexually experienced female migrants, about 10% of women in the entertainment establishments reported having sold sex, 30% having multiple sexual partners, and 40% having sex with men with multiple sexual partners. The rate of consistent condom use was less than 15% (*52*). The rates of prevalence of casual and commercial sex for female temporary migrants were found to be 14 and 80 times those for female nonmigrants, respectively. Female temporary migrants' higher unemployment rate and concentration in the service and entertainment sectors are keys to understanding such differences (*53*). Based on biological examinations, a study conducted among 505 female sex workers in Kunming, Yunnan Province revealed that the prevalence of chlamydia was 58.6%, that of gonorrhea was 37.8%, that of herpes simplex II was 65.1%, and that of syphilis was 9.5% (*54*).

Sexual behavior and HIV risks among "money boys"

The migrant subgroup known as "money boys" refers to young males who engage in same-sex sexual activities for economic survival. There are only a few summaries and commentaries on this topic (*55,56*). Mi and Wu quoted Zhang's study on MSM sex workers and estimated there were 380,000 such young male prostitutes in China (*55*). In a recent commentary in Cell Research, Zhang and Chu argue that the MSM sex trade deserves more attention and investigation (*56*). Meanwhile, anecdotal evidence suggests that these young prostitutes trade sex for money and drugs; some especially target Western foreigners. To the extent known, there is only one published English empirical study on HIV among these young male prostitutes (*57*). An ethnographic approach indicated that, compared to typical male migrants, young male prostitutes had a slightly better economic situation, rarely visited their hometowns, used alcohol less but drugs more, and had more knowledge about HIV and STIs, higher HIV/STI testing rates; and fewer HIV risk behaviors (*57*). However, young male prostitutes who were new to this work were often noted to lack knowledge about HIV and protection. Moreover, many of the young male prostitutes wanted STI testing since they perceived having such an infection as an immediate threat to

their vocation. About one-fifth of the young male prostitutes self-identified as heterosexuals and claimed their commercial sexual activities as purely "business transactions". This sub-group (heterosexual-identified young male prostitutes) continues to have heterosexual sexual encounters in their private lives (*57*). Based on the above ethnographic study, a quantitative survey of HIV risk and testing behaviors among 239 self-identified young male prostitutes and 100 typical male rural migrants in Shanghai was conducted. It found that, compared to typical male migrants, young male prostitutes were more likely to use alcohol, had more sexual partners and more casual sex partners, and were more likely to engage in other sexual risks. Both groups had substantial misconceptions about HIV/AIDS and reported low rates of condom use, regardless of who their sexual partners were (*58*). Moreover, the young male prostitutes were likely to be the victims of sexual violence at the hands of their clients. More than half of these prostitutes had been tested for HIV and 3% self-reported to be HIV-positive, whereas only 1% of the typical male migrants had ever been tested and all self-reported to be HIV-negative (*58*). Infection with other sexually transmitted diseases was also reported by young male prostitutes (*58*). Moreover, about 20% of these prostitutes self-identified themselves as heterosexual instead of homosexual man. They had female sex partners while providing commercial sex to male clients (*59*). Clearly, young male prostitutes play an important role in HIV transmission in China.

Implications for HIV/AIDS prevention and intervention

As demonstrated above, migrants from rural areas are a major part of the migratory population in China and are living and working in marginal and vulnerable situations within cities. This could be a particularly important concern with regard to public health and particularly the prevention and control of HIV/AIDS among the migrant population, which is an emerging public health challenge in China. The migratory status and unstable lifestyle of migrants hinders efforts to reach them with HIV/AIDS prevention and intervention programs. Yet ignorance could be disastrous to the migrant population, which may not only serve as a huge reservoir for an HIV pandemic but may also serve as a bridge for spread of the HIV epidemic between rural and urban populations. This is particularly relevant to China because the majority of identified HIV-infected individuals have been rural residents, among whom the heterosexual transmission of HIV is rapidly increasing. Therefore, a concerted effort should be made to control HIV/AIDS within as well as beyond the migrant population.

Careful examination of migrant society may prove helpful when designing and implementing HIV/AIDS-

related prevention and intervention programs. There is no doubt that migrants are predominantly young adults who are sexually active. Due to long-term separation from family members, loneliness, the strain of work and daily life, lack of entertainment facilities, and lack of essential knowledge regarding HIV/AIDS, migrants are more likely to engage in risky behaviors such as buying commercial sex and engaging in unprotected sex. In addition, their fundamental social norms and rules that previously guided their behavior in their own villages are often abandoned or play only a minimal role in their behavior in a new and more open society. Therefore, they are vulnerable to negative norms or beliefs and engage in risky behaviors in certain circumstances. Moreover, the perception and the fact that they are socially, culturally, and economically marginalized in the urban community makes them more susceptible to HIV infection. However, studies have suggested that HIV/STD prevention activities are accepted among rural-to-urban migrants (*60*).

Thus, HIV/AIDS prevention and intervention programs that are socio-economically and culturally tailored to the migrant population are urgently needed. Important suggestions or recommendations can be drawn from the current understanding of this special population. First, such programs must provide necessary and sufficient information about HIV/AIDS and healthy behaviors in easily understood formats and languages. Such information must also be disseminated or propagated by appropriate means that are particularly suited to the living and working conditions of migrants. Second, these programs or projects might include sessions that are intended to instruct migrants in how to deal with various barriers to access to health services in cities. Third, these programs must take advantage of the social network of the migrant population and involve close communication between people from both migrant and host city communities. Of particular help would be for project organizers, investigators, or implementers to consult and negotiate with leaders of a wide range of groups that represent various opinions and interests within and beyond the migrant society. Fourth, creation of a "migrant-friendly" atmosphere or environment during the implementation of these programs is crucial. The understanding and support of the host city community is also essential for successful implementation of any HIV/AIDS prevention and intervention project. Fifth, these programs may consider the possibility of providing appropriate entertainment facilities. Last, successful implementation of any HIV/AIDS prevention and intervention program is only achievable through close cross-cultural cooperation between people from various related communities or societies.

Acknowledgements

References

1. Ministry of Health of China, UNAIDS, WHO. 2005 update on the HIV/AIDS epidemic and response in China. Beijing: Ministry of Health, Jan 24, 2006.
2. Chinese Center for Disease Control and Prevention (CDC) and National Center for HIV/AIDS and STD Control and Prevention (NCAIDS): Annual Report on STDs in China in 2002.
3. People's Daily Online, "More women suffer from HIV/AIDS in China." June 4, 2007.
4. Qi X. HIV/AIDS epidemiology and China five year action plan and research. In: Proceedings of the first Sino-US conference on research and planning in AIDS-related areas. Beijing, China, 2002.
5. China National Bureau of Statistics. Characteristics of Chinese rural migrants: 2000. Beijing, China, 2002.
6. He N, Detels R, Chen Z, Jiang Q, Zhu J, Dai Y, Wu M, Zhong X, Fu C, Gui D. Sexual behavior among employed male rural migrants in Shang Hai, China. AIDS Educ Prev 2006;18:176-186.
7. He N, Detels R, Zhu J, Jiang Q, Chen Z, Fang Y, Zhang X, Wu M, Zhao Q. Characteristics and sexually transmitted diseases of male rural migrants in a metropolitan area of Eastern China. Sex Transm Dis 2005;32:286-292.
8. Hong Y, Stanton B, Li X, Yang H, Lin D, Fang X, Wang J, Mao R. Rural-to-urban migrants and the HIV epidemic in China. AIDS Behav 2006;10:421-430.
9. Hu Z, Liu H, Li X, Stanton B, Chen X. HIV-related sexual behaviour among migrants and non-migrants in a rural area of China: role of rural-to-urban migration. Public Health 2006;120:339-345.
10. Li X, Fang X, Lin D, Mao R, Wang J, Cottrell L, Harris C, Stanton B. HIV/STD risk behaviors and perceptions among rural-to-urban migrants in China. AIDS Educ Prev 2004;16:538-556.
11. Liu H, Xie J, Yu W, Song W, Gao Z, Ma Z, Detels R. A study of sexual behavior among rural residents of China. J Acquir Immune Defic Syndr Hum Retrovirol 1998;19:80-88.
12. Zheng L, Zhu J, Tian P, Chen Y, Song Y, Chen Y. Sexual behaviors and associated factors among unmarried female migratory workers in Guangzhou. Chinese Journal of Family Planning 2000;60:162-164.
13. Qiao X, Guo X, Zhang L, Mao A, Wang F, Liang Z, Xia S, Li J. Survey on moving status among HIV positives in Shanxi Province. Chinese Journal of AID & STDs 2000;6:265-267.
14. Huang C. Characteristics, impact and trend of the migration of rural labors in the 1990s in China. Popul Res 1998;22:8-14.
15. Zheng G, Hong W. Women in migration. Southern Population 1995;3:37-41.
16. Fan C. Impact of migrants on social and economic development. References for Economic Study 1998;57:19-25.
17. Lu Y, Zhao F. Economic analysis on migrants in China. Market and Demographic Analysis 1995;1:12-14.
18. Zhao M. Case study on floating population in metropolitan areas. Journal of Central Southern Financial and Economic University 1995;5:51-55.
19. Wang X. Control of crimes among migrants. Journal of Yunnan Public Security College 2001;1:1-4.
20. Xiao J. A review of study on crimes among migrants.

Journal of Jiangsu Public Security College 2001; 15:25-32.

21. Ding J, Yang H, Yang J, Weng J, Zhang B. Characteristics and control of migrant criminals in Shanghai. Popul Res 2001;25:53-58.

22. Ouyang X, Xie H. An exploratory examination of the problem of migrant population in current China. Journal of Xiang-Tan University 1996;2:57-63.

23. Ding Y. New characteristics of migrant population and causes of migration. References for Economic Study 1998;57:26-30.

24. Zhang W, Li X, Ye L, Xie G, Hu Y. Analysis on current situation of migrant population in China. References for Economic Study 1998;57:2-18.

25. Sun C. Patterns of China's population mobility – their role and impact on the development of Shanghai. In: Villagers in the city: rural migrants in Chinese metropolises (Gransow B, Hanlin L, ed.). Central Translation and Compilation Press, Beijing, China, 2001; pp. 130-164.

26. Wuellner C. Labor migration in Shanghai – its characteristics and impacts on the urban labor market. In: Villagers in the city: rural migrants in Chinese metropolises (Gransow B, Hanlin L, ed.). Central Translation and Compilation Press, Beijing, China, 2001; pp. 195-218.

27. Yang H. Trend and projection of the development of migrant population in Shanghai. Population Journal 1995;2:31-35.

28. Gransow B. Global forces and working girls: new socioscapes in the Pearl River Delta. In: Villagers in the city: rural migrants in Chinese metropolises (Gransow B, Hanlin L, ed.). Central Translation and Compilation Press, Beijing, China, 2001; pp. 283-303.

29. Jin W. Living in the margin of cities – the living situation of floating peasants. Journal of Guangxi University for Nationalities (Philosophy and Social Science Edition). 2001;23:2-9.

30. Liu L. Presentation and self-perception of rural migrants in Chinese metropolises. In: Villagers in the city: rural migrants in Chinese metropolises (Gransow B, Hanlin L, ed.). Central Translation and Compilation Press, Beijing, China, 2001; pp. 95-129.

31. Cai F, Li P, Liu Q, Li Z, Han J, Xu L, Yu X. The evolution of labor market and the migration of peasant workers. China Rural Survey 1996;2:51-56.

32. Zhang W, Chen S, Han J. Impact and volume control of migrants in cities in China. Northwest Population 1999;3:77-80.

33. Huang G. Migrants in communities between urban and suburban areas. Journal of Hangzhou Teachers College 1998;1:119-124.

34. Luo D. Characteristics of migrants in Chengdu and transition of administration. Journal of Chengdu University (Social Science Edition) 2002;3:43-48.

35. Wu W, Wang H. Living in metropolitan areas: current status of housing for migrants in Beijing and Shanghai. Sociological Study 2002;3:236-254.

36. Zhang D. Marginal status of peasant workers in cities. Youth Study 2000;9:91-94.

37. Zhu C, Gu C, Zhang W. Analysis on the characteristics of migrants in China urban areas. Population Journal 2001;2:3-7.

38. Tang B. Dual marginal people: establishment and social impact of self-recognition of peasant workers in cities. Journal of South-Central University for Nationalities (Humanities and Social Science) 2002;22:63-65.

39. Gransow B, Li H. Villagers in the city: rural migrants in Chinese metropolises. Central Translation and Publication House, Beijing, China, 2001; pp. 3-94.

40. Hui D, Chen F, Cai L. Data of migrant children at age of 0-6 years and their vaccination status. Journal of Suzhou Medical College 2000;20:733-734.

41. Jin R, Bei Q, Sun X. Current status of vaccination among migrant children in Zhenzou. Journal of Henan Preventive Medicine 1995;6:349-350.

42. Liu M, Zhang X, Li X, Yang Y. Analysis on the situation of EPI administration of the floating population in Guiyang city. Chinese Journal of Vaccines and Immunization 1998;4:312-315.

43. Sun M, Liu D, Liu A, Liu D, Fan C, Miao L, Lu L, Zhang X. Investigation of immunization coverage rate of children living in floating population area and the affecting factors. Chinese Journal of Vaccines and Immunization 2002;8:19-22.

44. Chen J, Liang J, Jing S. EPI among migrant children in Guangzhou. Guangdong Anti-epidemic 1999;25:2-3.

45. Li X, Fang X, Lin D, Mao R, Wang J, Cottrell L, Harris C, Stanton B. HIV/STD risk behaviors and perceptions among rural-to-urban migrants in China. AIDS Educ Prev 2004;16:538-556.

46. Anderson AF, Qingsi Z, Hua X, Jianfeng B. China's floating population and the potential for HIV transmission: a social-behavioural perspective. AIDS Care 2003;15: 177-185.

47. Lin D, Fang X, Li X, Xu J, Liu H. HIV/AIDS prevention and intervention in Chinese migrants. Chinese Journal of AIDS & STDs 2005;11:158-160.

48. Liu H, Li X, Stanton B, Fang X, Mao R, Chen X, Yang H. Relation of sexual risks and prevention practices with individuals' stigmatising beliefs towards HIV infected individuals: an exploratory study. Sex Transm Infect 2005;81:511-516.

49. Hesketh T, Li L, Ye X, Wang H, Jiang M, Tomkins A. HIV and syphilis in migrant workers in eastern China. Sex Transm Infect 2006;82:11-14.

50. Detels R, Wu Z, Rotheram MJ, Li L, Guan J, Yin Y, Liang G, Lee M, Hu L, the National Institute of Mental Health (NIMH) collaborative HIV prevention trial group. Sexually transmitted disease prevalence and characteristics of market vendors in eastern China. Sex Transm Dis 2003;30:803-808.

51. Chen X, Yin Y, Gong X, Liang G, Zhang W, Poumerol G, Shi M, Wu S, Zhang G. Prevalence of sexually transmitted infections among long-distance truck drivers in Tongling, China. Int J STD AIDS 2006;17:304-308.

52. Yang H, Li X, Stanton B, Fang X, Lin D, Mao R, Liu H, Chen X, Severson R. Workplace and HIV-related sexual behaviors and perceptions among female migrant workers. AIDS Care 2005;17:819-833.

53. Yang X, Xia G. Gender, migration, risky sex, and HIV infection in China. Stud Fam Plann 2006;37:241-250.

54. Chen XS, Yin YP, Liang GJ, Gong XD, Li HS, Poumerol G, Thuy N, Shi MQ, Yu YH. Sexually transmitted infections among female sex workers in Yunnan, China. AIDS Patient Care STDS 2005;19:853-860.

55. Mi G, Wu Z. Male commercial sex workers and HIV/AIDS. Chinese Journal of AIDS & STDs 2003;9:252-253.

56. Zhang BC, Chu QS. MSM and HIV/AIDS in China. Cell Res 2005;15:858-864.

57. He N, Wong FY, Huang ZJ, Thompson EE, Fu CW, Ding YY. Substance use and HIV risks among male heterosexual and money boy migrants in Shanghai, China. AIDS Care 2007;19:106-115.

58. He N, Wong FY, Huang ZJ, Ding YY, Fu CW, Smith B, Young D, Jiang QW. HIV risks among two types of male migrants in Shanghai, China: money boys *vs*. general male migrants. AIDS 2007 (in press).

59. Wong FY, Huang ZJ, He N, Smith B, Ding YY, Fu CW, Young D. HIV risks among gay- and non-gay-identified migrant money boys in Shanghai, China. AIDS Care 2007 (in press).

60. Yang H, Li X, Stanton B, Fang X, Lin D, Mao R, Chen X, Liu H. Willingness to participate in HIV/STD prevention activities among Chinese rural-to-urban migrants. AIDS Educ Prev 2004;16:557-570.

Improving the quality of healthcare in Japan: A systematic review of procedural volume and outcome literature

Hiroaki Miyata[1],*, Noboru Motomura[2], James Kondo[3], Shinichi Takamoto[2], Toshihiko Hasegawa[4]

[1] *Department of Healthcare Quality Assessment, Graduate School of Medicine, University of Tokyo, Tokyo, Japan;*

[2] *Department of Cardiothoracic Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan;*

[3] *Healthcare Policy Institute, Tokyo, Japan;*

[4] *Department of Health Policy and Management, Nippon Medical School, Tokyo, Japan.*

SUMMARY   **Though some policies have been implemented based on volume-outcome relationships in Japan, no studies systematically reviewed volume-outcome research conducted in Japan. Original data used in this study were obtained from MEDLINE searches using PubMed or from searches of the *Ichushi* database and complemented with manual searches. Two investigators reviewed and scored 13 articles, using a standard form to extract information regarding key study characteristics and results. Of the 13 studies we reviewed, 11 studies sought to detect the effects of hospital volume on outcomes while 2 examined the influence of individual physician volumes. Of the 13 studies, 9 studies (69.2%) indicated a statistically significant association between higher hospital volumes and better health outcomes. No study documented a statistically significant association between higher volumes and poorer outcomes. Higher review score is considered to be associated with significant association. The definition of low volume differed widely in each of the studies we reviewed. The 95%CI of healthcare outcomes is considerable even in studies that revealed a significant difference between volumes and outcomes. Higher hospital volumes are thought to be associated with better aggregate healthcare outcomes in Japan. For this reason, minimal-case-number standards might be effective to some extent. However, volume alone is not sufficient to predict the quality of healthcare. In addition, outcome-based evaluation might also be needed.**

Key Words: Volume-outcome, systematic review, healthcare, procedural volume, evaluation

Introduction

In 2002, the Japanese Ministry of Health, Labor, and Welfare set minimal standards by relating surgical fees to hospital procedure volumes (*1*). This policy might be based on the hypothesis that outcomes of complex healthcare procedures are better when done by providers or hospitals that perform them more frequently. For cardiac surgery specifically, those medical institutions that had an annual cardiac surgery procedural volume of fewer than 100 cases had their medical fees lowered by 30%. However, many stakeholders raised objections to these practices. One of the reasons for their objections stemmed from the fact that most medical institutions had their fees lowered; *i.e.* two thirds of Japanese medical institutes conducted fewer than 100 procedures per year (*2*). Though these standards were temporarily suspended starting in 2006, the Japanese Ministry of Health, Labor, and Welfare is still considering whether regionalization would be appropriate when considering hospital volumes.

Additionally, the Japanese Government updated medical practice laws in June of 2006. Each local

government was, starting in April 2007, given the power to require medical centers to submit and release "certain information" that would be considered useful to patients who are choosing a hospital (*3*). As of January 2007, this "certain information" included hospital procedural volumes but few outcome indicators such as operative mortality or morbidity rates. However, "certain information" could come to include outcome indicators similar to those used in public reporting in New York State (*4,5*). Examining whether hospital volume is information that should be revealed is crucial, as well as determining its accuracy.

Measuring and understanding the association between surgical volume and outcomes in the delivery of health services has been the focus of much research in the United States since the 1980s (*6,7*). Recently, two systematic reviews suggested that high volume is associated with better outcomes but that the degree of this association varies greatly (*8,9*). As the complications included in these findings are partly due to methodological shortcomings in many studies, a rigorous examination of the proposed volume-outcome association is extremely crucial. In addition, no studies have systematically reviewed volume-outcome research conducted in Japan. This study set out to conduct a systematic review of the research evidence linking volume and outcome in Japan, to summarize and describe the methodological rigor of the existing literature, and to examine the research and policy implications of these findings.

Materials and Methods

The original data for this review were identified by searches of MEDLINE using PubMed and by those of the Ichushi (Japana Centra Revuo Medicina) database. In addition, experts were contacted about missed studies. Articles identified were those investigating the association between hospital (or individual surgeon) procedural volume and outcomes from 1 January to 30 March 2007. The search terms used were 'volume (*syoureisuu*)', 'outcome (*tiryouseiseki*)', 'frequency', 'outcome assessment', 'regionalization', 'Japan' and 'Japanese'. Papers written in either English or Japanese were reviewed. Only studies on Japanese populations living in Japan were included. Instances of multiple publications from the same database were excluded, with only the most complete publication selected.

Two of the authors scored each article independently using an IOM scoring system regarding volume-outcome studies (*9*). Reviewers were not blinded to journal, authors, or findings. Any discrepancies were resolved by discussion. Quality scores were summed across all 10 criteria for each study. The maximum possible total score was 18. Higher scores reflect an increasing likelihood of the study's ability to discern a generalizable conclusion about the nature and extent

of the relationship between volume and outcome (Appendix).

A study was assigned one point if the sample was representative of the general population of all patients who might receive the treatments examined in the study. A study was assigned two points if it included 50 or more physicians and 20 or more hospitals. If only one of these criteria was met, the study was assigned one point. No points were assigned if neither criterion was met. In many studies authors reported the number of hospitals in their sample but not the number of treating physicians. In these instances, the number of physicians was estimated by assuming it would be at least equal to the number of hospitals. If the total sample size was 1,000 patients or more, the study was assigned one point. A study was assigned 2 points if the total number of adverse events was greater than 100, one point if it was 21-100, and no points if it was 20 or less.

A study was assigned no points if the study assessed the relationship between outcome and either hospital or physician volume. If both were assessed separately, the study was assigned one point. If the joint relationships of hospital and physician volume were assessed independently in a multivariate analysis, the study was assigned 2 points. Finally, if a study examined both of these, in addition to another important component of the care process, it was assigned 3 points. If the appropriateness of patient selection was not addressed, it was assigned no points. If appropriateness was measured, 1 point was assigned. If it was measured and taken into account in the analysis of the volume-outcome relationship, the study was assigned 2 points.

If the volume was analyzed in only 2 categories, the study was assigned no points. If more than 2 categories were assessed, or if volume was treated as a continuous variable, the study was assigned 1 point to credit a more sophisticated assessment of a possible dose-response relationship. In considering the various ways in which outcomes might be risk-adjusted, a study was assigned no points if no risk-adjustment was done at all. If data from insurance claims, hospital discharge abstract databases, or other sources of administrative data were used, the study was assigned 1 point. If data from clinical sources (*e.g.*, medical records or prospectively designed clinical registries) were used for risk-adjustment, the study was assigned 2 points. If clinical data were used in a logistic regression model that demonstrated good calibration by the Hosmer-Lemeshow test and good discrimination (by a C-statistic of 0.75 or greater), the study was assigned 3 points. If specific clinical processes of care were not measured, no points were assigned. If a single process was measured and its impact on risk-adjusted outcomes assessed, 1 point was assigned. If 2 or more such processes were measured and evaluated, 2 points were assigned. Finally, if death was the only outcome evaluated, no points were assigned. If other adverse

outcomes in addition to mortality were assessed, 2 points were assigned.

Results

This systematic review identified 13 articles (*10-22*). As a result of evaluating each article that studied more than one procedure as more than one study, these studies were found to cover 13 clinical topics. The methodological characteristics of the 13 articles are described in Tables 1-1 and 1-2. All studies identified were published after 2001.

With regard to the representative nature of the sample, 6 studies were considered using a representative database. Four studies were based on the Osaka Cancer Registry. The Osaka Cancer Registry has been operating since December 1962, covering Osaka Prefecture and its population of 8.8 million (*15*). Cancer incidence data in Osaka have been reported in 'Cancer incidence in five continents' volumes III to VIII (*23*). The Japanese coronary intervention study (*22*) consisted of a random sample (10%) of PCI procedures by a 2-step sampling process. First, 144 PCI facilities were randomly selected with stratification by hospital annual volume. Secondly, all PCI procedures were recorded at facilities performing 1-150 PCI per year. For the 2002 annual survey of the Japanese Society of Anesthesiologists (JSA) (*16*), 1,987,988 patients were registered from 704 training hospitals certified by the JSA. The 1996 National Patient Survey and 1996 National Hospital Survey (*19*) are 70% stratified random sampling surveys. The response rate in these studies was 100%.

With regard to the study sample size, 7 studies had sample sizes that exceeded 1,000, included 20 or more hospitals, 50 or more physicians, and more than 100 adverse events. With regard to the primary outcome, 11 studies reported mortality rates and 2 studies reported the length of hospital stays. Four studies measured outcomes besides death alone.

Among the 13 studies reviewed, 11 studies attempted to detect the effects of hospital volume on outcome whereas 2 examined the influence of individual physician volumes. No study examined both hospital and individual physician volumes or explored their joint effects. Additionally, no study measured the appropriateness of patient selection. Seven studies examined clinical processes of care, such as surgery type, surgical back up, ADL support, and additional treatment.

Nine studies used a multiple volume index and 2 studies used a two-category volume index. With regard to risk adjustment, 2 studies performed no adjustment while 3 studies used administrative data to adjust for some combination of age and sex. Though eight studies used clinical data in their risk-adjustment, no study reported a robustly discriminating and well-calibrated

risk model.

Of the 13 studies, 9 studies (69.2%) indicated a statistically significant association between higher hospital volumes and better health outcomes (Tables 2-1 and 2-2). Though the other 4 studies did not report a statistically significant association, their results indicated that higher hospital or physician volumes tended to be related to better health outcomes. No study documented a statistically significant association between higher volumes and poorer outcomes.

Higher review score is considered to be associated with significant association between procedural volume and healthcare outcomes. Regarding review scores, 3 of the 4 studies that did not indicate a statistically significant association between higher volume and better outcome received fewer than 3 points. Of those, 2 studies used results from a single hospital survey with patient populations of around 100. Another study was a retrospective survey regarding members of an academic association and did not state the patient sample size. Though the study regarding patients with AMI who had undergone PCI in 1997 (*22*) had earned a high score, with clinical risk-adjustment and sufficient sample size, results of the study did not indicate a statistically significant association. Another study involving AMI did not report a statistically significant association between the hospital volume and a shorter length of stay in 1998. However, the same study indicated a significant association between the two in 2002. Authors suggested that one of the reasons for their findings might have been that the use of clinical pathways as standardized protocols for management of patients with AMI had only been recently introduced to a high-volume hospital.

The definition of low volume in each study examined differed widely. Though definitions of low volume regarding ovarian (0.3 average per year; 84.5% of hospitals fall under the low volume category) and uterine cancer (0.6 average per year; 84.2% of hospitals fall under the low volume category) are very low, those concerning stomach cancer (16 average per year; 83% of hospitals fall under the low volume category) and AMI (7.3 average per year; 34.1% of hospitals fall under the low volume category) are relatively high. In terms of healthcare outcomes, the 95% confidence intervals were relatively high even in studies that indicated significant differences between hospital volumes and better outcomes.

Discussion

Results revealed that 9 of 13 Japanese studies claimed that all Japanese studies indicated a statistically significant association between higher hospital volumes and better health outcomes. No study showed a statistically significant association between higher volumes and worse outcomes. In Japan, higher hospital

Table 1-1. Methodological characteristics of volume-outcome studies 1

	1. Representativeness of sample		2. Number of hospitals or doctors		3. Total sample size (cases)		4. Number of adverse events		5. Unit of analysis	
	0 *Not* 1 *Representative*		0 *H < 20 and MD < 50* 1 *H < 20 or MD < 50* 2 *H ≥ 20 and MD ≥50*		0 *< 1,000* 1 *≥ 1,000*		0 *≤20* 1 *21-100* 2 *>100*		0 *Hospital or MD* 1 *Both separately* 2 *Both together* 3 *Both +*	
Saika, 2007	1	The Osaka cancer registry	2 171 hospitals		1 2,819		2 >100		0 Hospital only	
Tsuchihashi, 2004	1	Japanese coronary intervention study	2 129 hospitals		1 2,491		2 >100		0 Hospital only	
Nabae, 2003	1	National patient survey and the national hospital survey	2 1,399 hospitals		1 4,576		2 >100		0 Hospital only	
Ioka, 2004	1	The Osaka cancer registry	2 207 hospitals		1 3,523		2 >100		0 Hospital only	
Ioka, 2005	1	The Osaka cancer registry	2 89 hospitals		1 1,937		2 >100		0 Hospital only	
Nomura, 2003	1	The Osaka cancer registry	2 296 hospitals		1 15,413		2 >100		0 Hospital only	
Irita, 2004	1	Intraoperative critical incidents independent of the surgical site	2 704 hospitals		1 1,987,988		2 804		0 Hospital only	
Kinjo, 2004	0	Japanese coronary intervention study	1 25 hospitals		1 4,525		0 -		0 Hospital only	
Mitsuyasu, 2006	0	10 domestic national hospitals, 9 private hospitals	0 19 hospitals		0 827		0 -		0 Hospital only	
Haga, 2001	0	Six national hospitals in Japan that joined the E-pass study group	0 6 hospitals		0 902		0 18 in hospital death		0 Hospital only	
Abe, 2005	0	Surgeon members of the Japan pancreatic surgery club	2 148 hospitals		0 -		0 two		0 MD only	
Fujita, 2002	0	One University hospital	0 21 MDs		0 136		0 two		0 MD only	
Fujino, 2002	0	One University hospital	0 -		0 107		0 nine		0 MD only	

Table 1-2. Methodological characteristics of volume-outcome studies 2

(to be continued)

	6. Appropriateness of patient selection	7. Volume	8. Risk adjustment	9. Clinical processes of care	10. Outcomes	Total Score
	0 *Not measured* 1 *Measured separately* 2 *Measured and analysed separately*	0 *Two categories* 1 *Multiple*	0 *None* 1 *Admin only* 2 *Clinical data* 3 *Clinical +C > 0.75 and H/L test +*	0 *Not measured* 1 *One* 2 *2 +*	0 *Death only* 1 *Death +*	
Saika, 2007	0 Not measured	1 Continuous	2 Clinical data	1 Additional treatment	1 30-day mortality, 5-year survival	11
Tsuchihashi, 2004	0 Not measured	1 Three categories at terciles of annual volume	2 Clinical data	1 Surgical backup	1 In hospital mortality or CABG	11
Nabae, 2003	0 Not measured	1 Continuous	1 Admin only	1 ADL support	1 In hospital mortality, ength of stay	10
Ioka, 2004	0 Not measured	1 Four categories	2 Clinical data	1 Surgery type	0 5-year survival	10
Ioka, 2005	0 Not measured	1 Four categories	2 Clinical data	1 Surgery type	0 5-year survival	10
Nomura, 2003	0 Not measured	1 Divided into 4 categories of hospital volume with almost equal size	1 Admin only	0 Not measured	0 5-year survival	8
Irita, 2004	0 Not measured	1 Five categories	0 None	0 Not measured	0 7 day mortality	7
Kinjo, 2004	0 Not measured	0 Two categories	2 Clinical data	2 Coronary angiography before discharge, CABG before discharge	0 Length of hospital stay	6
Mitsuyasu, 2006	0 Not measured	0 Two categories	2 Clinical data	1 Transfer to another hospital length of hospital stay	0 Mean hospital charges,	3
Haga, 2001	0 Not measured	1 Three categories	2 Clinical data	0 Not measured	0 In hospital mortality	3
Abe, 2005	0 Not measured	1 Three categories	0 None	0 Not measured	0 Incidence of all arterial hemorrhages	3
Fujita, 2002	0 Not measured	0 Two categories	1 Admin	0 Not measured	1 Mortality and morbidity	2
Fujino, 2002	0 Not measured	0 Two categories	2 Clinical data	0 Not measured	0 Pancreatic leakage related mortality	2

Table 2-1. Summary table of the association between procedural volume and healthcare outcome in Japanese studies 1

Study	Field	Population	Time period	Patient #	MD #	Hospital #	Unit of Analysis	Primary outcome	Risk adjustment data source	Definition of low volume	Method	Volume-outcome results	Discussion regarding volume-outcome
Saika, 2007	Lung cancer, Stomach cancer	The Osaka Caner registry	1986-1995	2,819 (lung)	-	122 (lung)	Hospitals	30th day up to the 1830th day (5-year period mortality)	Clinical	Continuous	Logistic regression	In the logisticlogistic regression nalysis, the surgical volume index was positively associated with the survival of patients at every point during 5 years highest on the 90th day.	Data indicates that a surgical volume influence on stomach and lung cancer survival over 5 years and appears more prominently between 60th-120th days after surgery.
Tsuchihashi, 2004	Patients with AMI who had undergone PCI were identified	Japanese Coronary Intervention Study	1997	2,491	-	129	Hospitals	In hospital mortality need for CABG	Clinical	Low average 7.3 [1-16] (34.1%), middle average 24.4 [17-55] (32.5%), high average 26.6 [56-370] (33.3%)	Cochran-Armitage test	Mortality or CABG (low 9.9%, middle 7.8%, high 8.1%; $p=0.66$), adjusted mortality or CABG Odd ratio (Low, 1.00, middle 0.76 (0.44-1.31), high 0.70 (0.43-1.23); $p=0.26$)	There was no significant relationship between hospital volume and in-hospital outcome.
Nabae, 2003	Gastric, Colon and Rectal Cancer surgery	National patient survey and the National hospital survey	1998	4,576 (gastric)	-	1,399 (gastric)	Hospitals	In hospitals mortality rate, Length of stay	Admin	Gastric cancer 1-3 per month (68.6%), Colon cancer 1-2 per month (71.3%), Rectal cancer 1-2 per month (80.1%)	Logistic regression and multivariable regression model	In hospital mortality (Gastric: OR 0.88 $p<0.01$, Colon: OR 0.99 $p=0.80$, Rectal: OR 1.06 $p<0.35$), Length of Stay (Gastric β -019 $p<0.001$, Colon β -0.037 $p<0.001$, Rectal β -0.036 $p<0.001$)	In Gastric cancer surgery both in-hospital mortality and LOS were significantly associated. In Colon and Rectal cancer only LOS was significantly associated.
Ioka, 2004	Ovarian cancer	The Osaka cancer registry	1975-1995	3,523	-	207	Hospitals	5-year survival	Admin	High: average 8.8 (1.9%), medium: average 4.0 (4.8%), low: average 2.0 (6.7%), very low: average 0.3 (84.5%)	Cox regression model	High→very low 5-year survival (55.0%, 46.2%, 34.2%, 22.3%), Adjusted Hazard ratio (1.0, 1.1 [0.9-1.3], 1.4 [1.2-1.6], 1.6 [1.4-1.9])	After adjustment for age and other variables using the cox regression model, the hazard ratio correlated positively with hospital volume ($p<0.01$).
Ioka, 2005	Uterine cancer	The Osaka cancer registry	1990-1997	1,937	-	89	Hospitals	5-year survival	Clinical	High: average 28.8 (2.2%), medium: average 22.4 (2.2%), low: average 5.7 (10.1%), very low: average 0.6 (84.2%)	Cox regression model	High →very low 5-year survival (77.6%, 71.4%, 62.7%,45.7%), Adjusted Hazard ratio (1.0, 1.3 [1.0-1.5],1.3 [1.1-1.5], 2.0 [1.9-3.3])	Cox regression model, patient receiving care in very low, low, or medium-volume hospitals were found to have higher risk of death than patients receiving care in high volume hospitals.
Nomura, 2003	Stomach cancer	Osaka cancer registry	1990-1994	15,413	-	296	Hospitals	5-year survival	Admin	Very low: average 16 [1-84] 83%, low: average 148 [96-223], medium average: 298 [231-421], High: average 549 [487-644]	Cox's Proportional hazards model	5-year survival high→very low Localized cancer (84% [82-86], 86% [83-88], 82% [79-84], 76% [72-79]), Regional cancer (43% [39-47], 47% [43-51], 41% [37-45], 24% [21-28]), Adjacent cancer (12% [8-16], 13% [9-17], 12% [8-17], 5% [3-8]), Distant cancer (4% [2-7], 4% [2-7], 2% [1-4], 2% [1-4])	Positive relationship in localized, regional, adjacent cancer. Not clear in the case of the distant group.
Irita, 2004	Intraoperative critial incidents independent of the surgical site	2002 annual survey conducted by the subcommittee on surveillance of anesthesia-related critical incidents.	2002	1,987,988 -	-	704	Hospitals	7-day mortality	None	Fewer than 1,000: 62 (9.1%), 1,000-1,999: 204 (29.0%), 2,000 3,999: 288 (40.1%), 4,000-5,999: 110 (15.6%), more than 000: 40 (5.7%)	Ch-square test and fisher test	Mortality rate: fewer than 1,000: 14.89% [8.48-21.3], 1,000-1,999: 5.88%[3.05-4.67], 2,000-3,999: 5.97% [3.19-4.57], 4,000-5,999: 4.48% [3.20-4.88], more than 6,000: 2.99% [2.19-4.05]	Surgical volume was shown to affect mortality independent of the surgical site

Table 2-2. Summary table of the association between procedural volume and healthcare outcome in Japanese studies 2

(to be continued)

Study	Field	Population	Time period	Patient #	MD #	Hospital #	Unit of Analysis	Primary outcome	Risk adjustment data source	Definition of low volume	Method	Volume-outcome results	Discussion regarding volume-outcome
Kinjo, 2004	Acute myocardial infarction	Osaka Acute Coronary Insufficiency Study	1998-2003	4,113	-	25	Hospitals	Length of hospital stay	Clinical	AMI case load >50 patients/year, Performance of PCI> 200 procedures/year	Multiply regression	AMI case load >50 patients/year -0.123 $p=0.021$, Performance of PCI> 200 procedures/year -0.114 $p=0.034$	With regard to time trends, admission to a high-Volume hospital was not a predictor of a shorter hospital stay but was significantly associated in 2002.
Mitsuyasu, 2006	Total hip arthroplasty (THA), Total arthroplasty (TKA)	10 domestic national hospitals, 9 private hospitals	2001-2003	827 (THA)	-	19	Hospitals	Mean hospital charge, Length of hospital stay	Clinical	HA: High 45-151 (69.5%), Low <45 (30.5%), TKA: High 40-160 (62.3%), Low <40 (37.7%)	Multiply regression	Mean total charge: THA < 0.001, TKA =0.045. Mean LOS: THA <0.001, TKA < 0.001. Preoperative mean LOS: THA <0.001, TKA <0.001	Hospital surgical case volume has significant effects on the total length ofstay for both THA and TKA and on the total cost for THA
Haga, 2001	Consecutive patients who underwent elective gastrointestinal operations	Six national hospitals in Japan	1998-1999	902	-	6	Hospitals	In hospitals mortality rate	Clinical	Low <100 $n=1$, Medium 100 - <200 $n=3$, High ≥200 $n=2$	Linear regression	Mortality rate: High vs. Low Medium (5.7% vs. 18.4%, $p = 0.0350$)	Stepwise decrease in mortality was observed in accordance with the volume of operations
Abe, 2005	Pancreatic reconstruction	Surgeon members of the Japan pancreatic surgery club	2002	-	-	148	Hospitals	Incidence of all arterial hemorrhages	None	High volume hospital: PD was performed at least 60 times (2.7%), Low volume hospital: PD was performed fewer than 20 times (55%).	Student's t-test	Incidence of all arteria hemorrhage (low 3.2%±6.4, medium 2.6%±3.8, high 2.9%±2.3) incidence of delayed arterial hemorrhage (low 2.3%±6.1, medium 2.0%±2.9, high 1.9% ±1.9)	The incidences of all adverse events did not differ significantly
Fujita, 2002	Total Gastrectomy	One University hospital	1995-1998	136	21	1	MD	Mortality and Morbidity	Admin	Two surgeons who had done 30 or more operations were defined as high volume, the remaining 19 surgeons who had done fewer than 15 operations were defined as low volume surgeons	Multivariate analysis	high: low: mortality(0, 2: $p=0.17$), complications (7, 24: $p=0.04$)	Multivariate analysis identified surgeon's volume as a significant factor for development of postoperative complications
Fujino, 2002	Pancreatic leakage	One University hospital	1984-2000	107	-	1	MD	Leakage-related mortality	Clinical	High (>20 cases/study period), medium (>10-20 cases/study period)	Logistic regression	Leakage related mortality-univariate logistic regression analysis (Medium 13.3%, High 6.5% : $p=0.27$)	Surgeon volume did not have any significant influence

volume is believed to be associated with better health care outcomes in aggregate. Many other foreign systematic reviews have also suggested similar results (*8,24-28*). Since hospital procedural volumes attribute to physicians' skills, experienced interdisciplinary teams, well-organized care processes, and hospital facilities, they are a necessary factor when outcomes are considered. With regard to healthcare quality improvement, regionalization of medical centers based on hospital procedural volumes might be acceptable to some extent. The definition of low volume in the studies was very heterogeneous, so minimal volume standards need to be set carefully for each specialty. Moreover, regionalization has an impact not only on hospital quality, but also on patients' access, staffing of medical professionals, cooperation with other departments in the hospital, and healthcare expenditures.

Volume alone is not sufficient for prediction of outcome because there was a large variance in the results observed among individual centers, even in the studies that indicated a significant difference between volume and outcome. Not all high-volume providers have better outcomes, and not all low-volume providers have worse outcomes. In addition, hospital volume as well as a number of other parameters (namely, outcome monitoring, compliance with process measures, and appropriateness of patient selection for surgery) might be associated with better outcomes (*4,29*). Quality improvement in the healthcare field might not be achieved fully by only using the minimal volume standards. Evaluating and encouraging quality improvement based on healthcare outcomes might be another way of improving the quality of healthcare. Birkmeyer suggested three strategies for improving surgical quality based on performance: centers of excellence (selective contracting, financial incentives for patients, and public reporting to direct patients to the best hospitals or surgeons), pay for performance (improving quality at all hospitals by rewarding good performance with financial bonuses), and pay for participation (improving quality at all hospitals by underwriting clinical outcomes registries and quality-improvement activities) (*30*). These outcome-based evaluations need to satisfy two requirements: 1) detailed clinical data for risk adjustment (*30*) and 2) a large enough sample size for each hospital's outcome indicator (*31*). In Japan, however, clinical databases have not been established in most healthcare fields and discussion regarding risk-adjustment has not taken place. Ensuring a large enough sample size for each procedure may also be difficult because most medical centers belong to the very-low or low volume categories. Both minimal care standards and outcome-based evaluation might be effective to some extent as means of improving healthcare quality in Japan.

Several limitations should be noted. A negative publication bias may have existed to diminish the number of studies failing to report expected associations. In addition to the heterogeneous methods used in the studies, the number of procedures included in this review is limited. With regard to specific health policy recommendations, further detailed analysis is needed in each healthcare field.

References

1. Miyata H, Motomura N, Takamoto S. Regionalization of cardiac surgery in Japan I- Effect of procedural volume on outcome of CABG surgery. Kyobu-Geka 2007;60:334-343. (in Japanese)
2. Miyata H, Kondo JM, Motomura N, Fushimi K, Takamoto S. Regionalization of cardiac surgery in Japan II. Estimating the multiple effect of regionalization in cardiac surgery. Kyobu Geka 2007;60:418-425. (in Japanese)
3. Fifth revision of the Medical Service Law and similar laws, Japan http://www.mhlw.go.jp/topics/bukyoku/soumu/houritu/dl/164-4a.pdf (accessed May 28, 2007).
4. Shahian DM. Improving cardiac surgery quality-volume, outcome, process? JAMA 2004;291:246-248.
5. Burack JH, Impellizzeri P, Homel P. Public reporting of surgical mortality. Ann Thorac Surg 1999;68:1195-1200.
6. Luft HS, Bunker JP, Enthoven AC. Should operations be regionalized? The empirical relation between surgical volume and mortality. N Engl J Med 1979;301: 1364-1369.
7. Luft HS. The relation between surgical volume and mortality: an exploration of causal factors and alternative models. Med Care 1980;18:940-959.
8. Halm EA, Lee C, Chassin MR. Is volume related to outcome in health care? A systematic review and methodologic critique of the literature. Ann Intern Med 2002;137:511-520.
9. Institute of Medicine, Hewitt M. Interpreting the Volume-Outcome Relationship in the Context of Health Care Quality: Workshop Summary. National Academy Press, Washington DC, USA, 2000.
10. Abe H, Tsukada K, Takada T, Nagakawa T. The selection of pancreatic reconstruction techniques gives rise to higher incidences of morbidity: results of the 30th Japan Pancreatic Surgery Questionnaire Survey on pancreatoduodenectomy in Japan. J Hepatobiliary Pancreat Surg 2005;12:109-115.
11. Fujino Y, Suzuki Y, Ajiki T, Tanioka Y, Ku Y, Kuroda Y. Risk factor influencing pancreatic leakage and the mortality after pancreaticoduodenectomy in a medium-volume hospital. Hepatogastroenterology 2002;49:1124-1129.
12. Fujita T, Yamazaki Y. Influence of surgeon's volume on early outcome after total gastrectomy. Eur J Surg 2002;168:535-538.
13. Haga Y, Ikei S, Wada Y, Takeuchi H, Sameshima H, Kimura O, Furuya T. Evaluation of an estimation of physiologic ability and surgical stress (E-PASS) scoring system to predict postoperative risk: A multicenter prospective study. Surg Today 2001;31:569-574.
14. Ioka A, Tsukuma H, Ajiki W, Oshima A. Influence of hospital procedure volume on ovarian cancer survival in Japan, a country with low incidence of ovarian cancer. Cancer Sci 2004;95:233-237.
15. Ioka A, Tsukuma H, Ajiki W, Oshima A. Influence of

hospital procedure volume on uterine cancer survival in Osaka, Japan. Cancer Sci 2005;96:689-694.

16. Irita K, Kawashima Y, Tsuzaki K, Iwao Y, Seo N, Morita K, Sawa T, Sanuki M, Makita K, Kobayashi Y, Obara H, Oomura A. Surgical volume and mortality due to intraoperative critical incidents at Japanese Society of Anesthesiologists-certified training hospitals: an analysis of the annual survey in 2002. Masui 2004;53:1421-1428. (in Japanese)

17. Kinjo K, Sato H, Nakatani D, Mizuno H, Shimizu M, Hishida E, Ezumi A, Hoshida S, Koretsune Y, Hori M; Osaka Acute Coronary Insufficiency Study (OACIS) Group. Predictors of length of hospital stay after acute myocardial infraction in Japan. Jpn Circ J 2004;68:809-815.

18. Mitsuyasu S, Hagihara A, Horiguchi H, Nobutomo K. Relationship between total arthroplasty case volume and patient outcome in an acute care payment system in Japan. J Arthroplasty 2006;21:656-663.

19. Nabae K, Hayashi K, Shirokawa M, Hasegawa T, Hasegawa T. The effect of procedural volume on digestive cancer. Byouin Kanri 2003;40:313-324. (in Japanese)

20. Nomura E, Tsukuma H, Ajiki T, Oshima A. Population-based study of relationship between hospital surgical volume and 5-year survival of stomach cancer patients in Osaka, Japan. Cancer Sci 2003;94:998-1002.

21. Saika K, Ohno Y, Tanaka H, Hasegawa T, Tsukuma H, Oshima A. The trend of the effect of surgical volume up to 5 years after resection for stomach and lung cancer patients. IT Healthcare 2007;1:42-49.

22. Tsuchihashi M, Tsutsui H, Tada H, Shihara M, Takeshita A, Kono S. Volume-outcome relation for hospitals performing angioplasty for acute myocardial infarction-- results from the Nationwide Japanese registry-. Jpn Circ J 2004;68:887-891.

23. Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB, editors. Cancer incidence in five continents vol. VIII In: IARC Scientific Pub. No.155. International Agency for Research on Cancer; Lyon, France, 2002.

24. Holscher AH, Metzger R, Brabender J, Vallbohmer D, Bollschweiler E. High-volume centers - effect of case load on outcome in cancer surgery. Onkologie 2004;27:412-416.

25. Kalant N, Shrier I. Volume and outcome of coronary artery bypass graft surgery: are more and less the same? Can J Cardiol 2004;20:81-86.

26. Killeen SD, O'Sullivan MJ, Coffey JC, Kirwan WO, Redmond HP. Provider volume and outcomes for oncological procedures. Br J Surg 2005;92:389-402.

27. Nuttall M, van der Meulen J, Phillips N, Sharpin C, Gillatt D, McIntosh G, Emberton M. A systematic review and critique of the literature relating hospital or surgeon volume to health outcomes for 3 urological cancer procedures. J Urol 2004;172:2145-2152.

28. van Heek NT, Kuhlmann KF, Scholten RJ, de Castro SM, Busch OR, van Gulik TM, Obertop H, Gouma DJ. Hospital volume and mortality after pancreatic resection: a systematic review and an evaluation of intervention in the Netherlands. Ann Surg 2005;242:781-788.

29. Ferguson BT, Peterson ED, Coombs LP, Eiken MC, Carey ML, Grover FL, DeLong ER; for the Society of Thoracic Surgeons and the National Cardiac Database. Use of continuous quality improvement to increase use of process measures in patients undergoing coronary artery bypass graft surgery. JAMA 2003;290:49-56.

30. Birkmeyer NJ, Birkmeyer JD. Strategies for improving surgical quality--should payers reward excellence or effort? N Engl J Med 2006;354:864-870.

31. Dimick JB, Welch HG, Birkmeyer JD. Surgical mortality as an indicator of hospital quality: the problem with small sample size. JAMA 2004;292:847-851.

Dengue infection: A growing global health threat

Marisa Hemungkorn, Usa Thisyakorn*, Chule Thisyakorn

Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

SUMMARY	Dengue infection, one of the most devastating mosquito-borne viral diseases in humans, is now a significant problem in several tropical countries. The disease, caused by the four dengue virus serotypes, ranges from asymptomatic infection to undifferentiated fever, dengue fever (DF), and severe dengue hemorrhagic fever (DHF) with or without shock. DHF is characterized by fever, bleeding diathesis and a tendency to develop a potentially fatal shock syndrome. Consistent hematological findings include vasculopathy, coagulopathy, and thrombocytopenia. There are increasing reports of dengue infection with unusual manifestations that mainly involve cerebral and hepatic symptoms. Laboratory diagnosis includes virus isolation, serology, and detection of dengue ribonucleic acid. Successful treatment, which is mainly supportive, depends on early recognition of the disease and careful monitoring for shock. Prevention depends primarily on control of the mosquito vector. Further study of the pathogenesis of DHF is required for the development of a safe and effective dengue vaccine.

Key Words: Dengue virus, dengue fever, dengue hemorrhagic fever, dengue shock syndrome, DF, DHF, DSS

Introduction

Dengue hemorrhagic fever (DHF) is an acute febrile illness found mainly in children. It is characterized by fever, bleeding diathesis, and a tendency to develop a potentially fatal shock syndrome. The disease is a major public health concern in South and Southeast Asia, the Western Pacific, and Central and South America and is now being reported in other tropical regions. It is one of the leading causes of hospitalization and death in children in countries where dengue disease is prevalent (*1*).

Epidemiology

Dengue is one of the most infectious human viral diseases transmitted by arthropod vectors. Annually, there are an estimated 50-100 million cases of dengue fever (DF) and 250,000 to 500,000 cases of DHF in the world. Over half of the world's population lives in areas at risk of infection. The resurgence of DF

**Correspondence to:* Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand;
e-mail: fmeduty@md2.md.chula.ac.th

and the emergence of DHF is due to unprecedented population growth, unplanned and uncontrolled urbanization, increased air travel, the lack of effective mosquito control, and the deterioration of public health infrastructure (*2*).

The disease's etiologic agents include all four dengue serotypes, which belong to the genus flavivirus of the family *Flaviviridae*. Primary infection with a particular dengue serotype confers long-lasting immunity for that serotype (homotypic immunity). Immunity to other dengue serotypes (heterotypic immunity) lasts for a few months, after which patients are susceptible to heterotypic infection. The principal vector is the mosquito, *Aedes aegypti*, which largely breeds indoors in clean water, and mainly in artificial water containers, and feeds on humans during the daytime.

Extensive epidemiological studies in Southeast Asia have shown that DHF occurs when two or more dengue serotypes are simultaneously endemic or sequentially epidemic and where ecological conditions favor efficient virus transmission via the mosquito vector. Serological studies demonstrate that there is an association between DHF and a secondary antibody response in most cases. These epidemiological and serological observations clearly link DHF to individuals who have had previous dengue infection or alternatively

have acquired maternal dengue antibody. Nisalak A *et al*. reviewed dengue virus incidence from 1973 to 1999 in Bangkok and demonstrated that all four dengue serotypes can be found circulating in any one year with one predominant serotype emerging and re-emerging as the cause of the epidemic over time. The authors concluded that the pathogenesis of DHF is complex and a product of host determinants, dengue serotype, and environmental factors (*3*).

Dengue virus infection in humans causes a spectrum of illnesses ranging from inapparent or mild febrile illness to severe and fatal hemorrhagic disease. Vertical transmission of dengue virus from mother to child has also been reported (*4*). The severity of DF manifestations increases with age. Infants and children with DF have symptoms ranging from an undifferentiated fever to mild febrile illness, sometimes associated with a rash. Older children and adults frequently suffer a more severe form with the triad of high fever, pain in various parts of the body, and a maculopapular rash. The infection is rarely fatal.

DHF is considered a distinct disease characterized by increased vascular permeability leading to hemoconcentration and dengue shock syndrome (DSS). DHF mostly affects children under 14 years of age and causes significant mortality (*1,5,6*). There is a strong association between good nutritional status and an increased risk of developing DSS. DHF and DSS are rarely seen in children with severe malnutrition (*7*).

Anecdotal records indicate that rare cases of DHF were seen in children with AIDS, while there was no difference in dengue seroprevalence between HIV-infected and healthy children. Further study and clarification is needed to determine whether the protective effect of immune suppression in HIV-infected persons prevents them from acquiring severe dengue disease (*8*).

Pathophysiology and Pathogenesis

The pathophysiological abnormality of DHF is an acute increase in vascular permeability without an inflammatory response, ultimately resulting in hypovolemic shock. Supporting evidence of plasma leakage includes serous effusions found at autopsy, pleural effusions and ascites on chest and abdominal roentgenograms (Figure 1), hemoconcentration, and hypoproteinemia. Immunological response plays a central role in disease pathogenesis since there is little or no viable virus in the circulation during the occurrence of increased vascular permeability, lending further credibility to the position that these events are mediated by processes not directly related to infection but rather to mediators such as cytokines. Elevated levels of cytokines and other markers of activated T cells support the role of cytokines in increased capillary permeability (*9-11*). Increased capillary permeability

may be due to gaps in the endothelium, and a recent study suggests that endothelial cells are a target for dengue virus infection and dengue virus-induced IL-6 and IL-8 production by endothelial cells, which may contribute to the increased capillary permeability (*12,13*). An association between cytokine-related gene expression levels and dengue disease severity has been demonstrated. This might serve as a predictor of dengue disease activity, leading to a proper therapeutic plan (*14*).

Activation of the complement system with profound depression of C3 and C5 levels in serum and the formation of immune complexes are found in all cases. The peak in complement activation and the presence of C3a and C5a anaphylatoxins coincide with the onset of shock and plasma leakage. Levels of C3a correlate closely with disease severity (*15*).

"Immune enhancement" of virus infection has been proposed in the pathogenesis of DHF. Dengue virus shows enhanced replication in human and simian peripheral blood leukocytes (most likely monocytes) in the presence of subneutralizing concentrations of specific antibody (*16,17*). Halstead proposed that an immune elimination response, probably mediated by T-lymphocytes, activates these dengue-infected monocytes to release a variety of factors that cause hemorrhage and shock. These include vascular permeability factor, complement activating factors, and

Figure 1. Chest roentgenogram indicating right pleural effusion in a patient with dengue hemorrhagic fever.

thromboplastin (*18-20*).

Other hypotheses suggest that DHF results from infection by a more virulent serotype or strains within serotypes of the virus. DHF has been diagnosed in patients with primary dengue infection that lack pre-existing dengue virus antibodies. Molecular characterization of the dengue virus has suggested that genetic variation between strains may be correlated with clinical manifestation and epidemiological characteristics (*21*). Murgue *et al.* showed that the duration and magnitude of dengue viremia, which did not significantly differ between primary and secondary dengue infection, determine disease severity. Their results did not support the immune enhancement hypothesis (*22*). On the contrary, Sudiro *et al.* did not find a significant difference in maximal plasma viral RNA levels between children with DHF and those with DF (*23*). Vaughn *et al.* determined the duration and magnitude of dengue viremia in serial plasma samples by viral culture and showed that viremia during primary infection was prolonged compared to secondary infection. Their study also showed that the rate of virus clearance was faster in patients experiencing secondary infection than in those with primary infection and was faster in those with DHF than those with DF (*24*).

For the last thirty years, two hypotheses concerning the mechanism of DHF have been debated. Some evidence points to secondary infection or viral virulence. The most plausible explanation is a combination of both hypotheses. Examples of significance of both viral and immunologic factors in dengue pathogenesis come from key studies performed during dengue outbreaks. An investigation of the recent outbreak in Cuba showed that almost all cases of DHF/DSS are secondary DEN-2 infections in adults previously infected by DEN-1 during a 1977-1979 epidemic (*25*). This supports the immune enhancement hypothesis. However, other investigations provide additional interesting information. An outbreak in 1980 in Rayong, Thailand demonstrated that despite the high rate of DEN-1 infection, only DEN-2 virus was recovered from DSS cases, including pre-illness serum specimens from two DEN-1 immune children. A seroprevalence survey prior to the outbreak also revealed that DEN-1 antibodies were the lowest, and yet children with this type were unmistakably prone to developing DSS in comparison to other children (*26*). This suggests that viral factors play a significant role in severe cases. Another good example is the introduction of the Southeast Asian genotype of the virus into some countries in the Americas, *i.e.* Venezuela, Brazil, Columbia, and Mexico. While the native DEN-2 of these countries had not been known to cause DHF/DSS, invasion of Southeast Asian strains coincided with occurrence of some severe cases (*27*). In addition, confirmation of this finding comes from a report of an outbreak in 1995 in Peru due to native strains of DEN-2; this followed an epidemic of DEN-1

five years earlier in the same population. No cases of DHF/DSS were found (*28*).

Certain ethnic groups may be more susceptible or resistant to the dengue virus since DHF is more common in Southeast Asia than in Africa or the Americas. Blacks were found to be relatively resistant to DHF/DSS during the 1981 Cuba outbreak, and there is speculation about a "resistance gene" present in the African population (*29*). Further epidemiological studies are needed to evaluate the effect of immune enhancement with risk factors such as viral virulence, other environmental or infectious agents, genetic susceptibility, or unknown host factors.

Progress in dengue pathogenesis research has been partly hampered by a lack of animal models. The recent development of a mouse model simulating human disease should help accelerate research progress in the field (*30*).

Diagnosis

The incubation period of dengue infection is usually 4-7 days but can range from 3 to14 days (*31*). Clinical and laboratory criteria for the diagnosis of DHF as established by the World Health organization are as follows (*32*).

Clinical criteria

● Fever - acute in onset, high, continuous, lasting for 2-7 days.
● Hemorrhagic manifestations - a positive tourniquet test, petechiae, purpura, ecchymosis, epistaxis, bleeding gums, hematemesis, melena.
● Hepatomegaly - observed in 90-96 and 60 percent of Thai children and adults, respectively.
● Shock - a rapid, weak pulse with a narrow pulse pressure; hypotension with cold, clammy skin and restlessness.

In patients with DHF grade I, a positive tourniquet test is the only hemorrhagic manifestation, whereas spontaneous bleeding occurs in DHF grade II. Patients with circulatory failure (narrowing of the pulse pressure and a rapid and weak pulse) have DHF grade III. Patients in profound shock (no detectable blood pressure and pulse) have DHF grade IV. Grades III and IV DHF are also referred to as DSS. In the initial febrile period, flushing of the skin is common and a centrifugal maculopapular rash is less common. In the convalescent stage, a confluent petechial rash with round pale areas of normal skin is commonly seen.

Clinical manifestations of dengue infection vary with age as DSS is more common in children than in adults. Infants with dengue infection present more frequently with convulsions, diarrhea, rash, cyanosis, and splenomegaly (*33-35*).

Laboratory criteria

● Thrombocytopenia (platelet count < 100,000/mm^3)
● Hemoconcentration (hematocrit increased by > 20%)

These criteria provide conclusive diagnosis in 90 percent of patients. The presence of the first two or three clinical criteria with thrombocytopenia and hemoconcentration is sufficient to establish the diagnosis of DHF. The diagnosis is highly likely to have DHF when shock occurs with high hematocrit levels, except in patients with severe bleeding (*32*).

Other common laboratory findings are hypo-proteinemia, hyponatremia, and elevation of hepatic enzymes and blood urea nitrogen levels. Metabolic acidosis may be found in patients with prolonged shock. White blood cell count is variable, ranging from leukopenia to mild leukocytosis with an increase in the percentage of lymphocytes and the presence of atypical forms (*36-38*).

Hematological findings include vasculopathy, reduction of several coagulation factors, reduced platelet count, and platelet dysfunction (*39*). Thrombocytopenia could be caused by the virus reducing hematopoietic progenitor cell growth and a subsequent decrease in thrombopoiesis (*40*). Interaction of the virus with the platelets through IgM anti-platelet autoantibody has been demonstrated in dengue patients (*41*). Disseminated intravascular clotting can occur in all grades of dengue infection. However, only in severe cases and in those with prolonged shock is disseminated intravascular coagulopathy a cause of uncontrolled bleeding and death (*39*). The tendency toward bleeding should be monitored in any dengue patients since it may cause severe, uncontrollable hemorrhaging (*4,42*). The pathogenesis of bleeding in dengue patient is not fully understood. The extent of endothelial cells, coagulation, and fibrinolysis activation in children with dengue infection seems to be correlated with dengue disease severity (*43*). The d-dimer, a specific marker for cross-linked fibrin, is often used as a marker for DIC and is significantly correlated with dengue disease severity (*44*).

The etiological diagnosis of dengue infection can be confirmed by serological tests or by isolation of the virus from blood specimens. Virus isolation is easier during the early febrile phase (*3,45*). Enzyme-linked immunosorbent assay (ELISA) for dengue antibodies is an improvement over the previous hemagglutination inhibition assay for serological confirmation (*46*). Commercial kits based on a serological approach to dengue diagnosis are available and need further testing with regard to sensitivity and specificity before they can be recommended for routine use. Detection of viral RNA by reverse transcription polymerase chain reaction is a highly sensitive technique for the early diagnosis of dengue infection (*47*). A pilot evaluation of diagnostic values of ELISA and reverse transcription polymerase chain reaction from oral specimens has yielded promising results (*48*). Collection of oral specimens is less invasive and may be more acceptable.

Unusual Manifestations

There have been increasing reports of dengue infection with unusual manifestations including encephalopathy, encephalitis, and fulminant hepatitis. Patients with these manifestations tend to be younger and have a significantly higher mortality rate than those with the more common form of the infection (*49-57*). Implications of severe infection such as cerebral edema, acidosis, fulminant hepatic failure, and bleeding may lead to encephalopathy (*51,52*).

Occasionally, dengue viruses can cross the blood-brain barrier and lead to encephalitis (*50,52-55*). Neurological manifestations of dengue include alteration of consciousness, seizures, pyramidal tract signs, meningeal signs, and headaches. Cerebrospinal fluid (CSF) examination shows lymphocytic pleocytosis in 20 percent of patients while the presence of anti-dengue IgM antibodies in CSF is detected in few patients. Dengue antigens have been found in the brain in fatal cases, but pathological evidence of encephalitis is rarely seen (*50,52*). Magnetic resonance imaging reveals cerebral edema in most patients evaluated but rarely indicates encephalitis-like alterations (*54*). In endemic areas, dengue should be considered in patients who present with clinical features of encephalitis, regardless of whether classical manifestations of dengue are present (*55*).

Hepatocellular injury manifested by hepatomegaly, elevation in alanine aminotransferase, and mild coagulopathy are common in DHF and even in DF, although hepatomegaly is absent (*56*). Acute liver failure is a major cause of death. Virus culture, immunocytochemistry, and electron microscopy confirm that dengue virus replicates in the liver. Whether liver injury is a direct effect of virus replication or a consequence of host response to infection is still unclear (*57*).

In all cases of unusual findings or unusual manifestations of dengue infection, a search should be conducted for a coinfection that may modify the clinical presentation. Such a coinfection could result in missed or delayed diagnosis and treatment of dengue infections and possibly be misinterpreted as an unusual manifestation (*58*).

Treatment

Treatment of dengue infection is symptomatic and supportive. In most cases, early and effective replacement of lost plasma with fluid and electrolyte solutions, plasma, and/or plasma expander results

in a favourable outcome. A single high dose of methylprednisolone does not reduce mortality in severe DSS and is not required for conventional critical care (*59*). The outcome depends on early recognition of infection and careful monitoring. Serial determinations of platelet and hematocrit levels are essential for the early recognition and prevention of shock. In rare cases, blood products are required. Blood transfusion is indicated for patients with significant clinical bleeding mainly from the gastrointestinal tract. Fresh frozen plasma and/or platelet concentrate are required when consumptive coagulopathy causes massive bleeding. Persistent shock despite adequate fluids and a decline in the hematocrit level suggest significant clinical bleeding requiring prompt treatment. Disseminated intravascular coagulation occurs in cases with severe shock and may play an important role in the development of massive bleeding and irreversible shock. Coagulation tests should be monitored in all cases of shock to document the onset and severity of disseminated intravascular coagulation. Blood grouping and matching should be carried out as a routine precaution for every patient in shock.

The rate of fluid infusion needs to be carefully tailored according to the patient's vital signs, hematocrit, and urine output. In general, there is no need for fluid therapy beyond 48 h after the cessation of shock. Reabsorption of extravasated plasma takes place, manifested by a further drop in the hematocrit level after intravenous fluids have been halted. This drop may cause hypervolemia, pulmonary edema, or heart failure if more fluids are given. An extremely important point is that a drop in the hematocrit level at this stage not be taken as a sign of internal hemorrhaging. A strong pulse and blood pressure, with a wide pulse pressure and diuresis, are good vital signs. They rule out the likelihood of gastrointestinal hemorrhaging as is mostly found during the shock stage (*60*).

Post-mortem Findings

In DHF, the most frequent gross anatomical findings post-mortem are petechial hemorrhages especially of the mucosa of the gastrointestinal tract, atrophy of the thymus and an increase in extravascular fluid with effusions in serous cavities, increased weight of organs, and edema most commonly in the retroperitoneum. Microscopically, there is no vasculitis. There is widespread evidence of diapedesis of red blood cells around blood vessels and interstitial edema in all tissues of the body. In capillaries and precapillary arterioles, swelling of some endothelial cells suggests that functional alterations are accompanied by structural derangements. Evidence of intravascular thrombosis is seen in some cases. There are degrees of coagulative necrosis of hepatocytes, varying from scattered cells within liver lobules to submassive and massive

involvement. Necrotic areas contain cells identical to the Councilman bodies seen in yellow fever that are accompanied by activation of Kupffer cells (*61*).

Prevention

Prevention of DHF depends on the control of the mosquito vector by limiting its breeding places and treatment of stored water with larvicide. These measures against dengue are effective only with a high level of government commitment, education, and community participation (*2*).

An effective, safe, affordable vaccine against the dengue virus is not an immediate prospect since pre-existing heterotypic antibodies within the host increase the risk for DHF and DSS. An effective vaccine will have to offer protection against all 4 serotypes of the virus. Dengue vaccines under development include the first generation live attenuated tetravalent vaccine developed at Mahidol University in Thailand, a second-generation attenuated vaccine created by genetic engineering, and vaccines created using new molecular approaches (*62-65*).

DHF has exhibited geographical expansion in Thailand and other tropical countries, where maintenance of well-documented clinical, epidemiological, and virological descriptions of the syndrome is crucial. Biological and social studies are essential to the development of effective mosquito control, treatments such as medications to reduce capillary leakage, and a safe vaccine.

References

1. Thisyakorn U, Thisyakorn C. Dengue hemorrhagic fever. In: Textbook of Travel Medicine and Health. 2nd ed. (Dupont HL, Steffen R, eds.). B.C. Decker Inc., Hamilton, 2001; pp. 312-314.
2. Rigau-Perez JG, Clark GG, Reiter P, Sanders EJ, Vorndam AV. Dengue and dengue haemorrhagic fever. Lancet 1998;352:971-977.
3. Nisalak A, Endy TP, Nimmannitya S, Kalayanarooj S, Thisyakorn U, Scott RM, Burke DS, Hoke CH, Innis BL, Vaughn DW. Serotype-specific dengue virus circulation and dengue diseases in Bangkok, Thailand from 1973 to 1999. Am J Trop Med Hyg 2003;68:191-202.
4. Thaithumyanon P, Thisyakorn U, Deerojanawong J, Innis BL. Dengue infection during parturition complicated by severe hemorrhage and vertical transmission. Clin Infect Dis 1994;18:248-249.
5. Thisyakorn U, Thisyakorn C. Studies on flaviviruses in Thailand. In: Progress in Clinical Biochemistry: Proceedings of the 5th Asian-Pacific Congress of Clinical Biochemistry. September 29-October 4, 1991. Kobe, Japan. (Miyai K, Kanno T, Ishikawa E, eds.). Excerpta Medica, Amsterdam, 1992; pp. 985-987.
6. Thisyakorn U, Thisyakorn C. Diseases caused by arboviruses-dengue haemorrhagic fever and Japanese B encephalitis. Med J Aust 1994;160:22-26.
7. Thisyakorn U, Nimmannitya S. Nutritional status of children with dengue hemorrhagic fever. Clin Infect Dis 1993;16:295-297.
8. Srettakraikul K, Hemungkorn M, Pancharoen C,

Thisyakorn U, Thisyakorn C. Seroepidemiology of dengue virus infection in HIV-infected children in comparison to healthy children. (submitted 2007)

9. Rothman AL, Ennis FA. Immunopathogenesis of dengue haemorrhagic fever. Virology 1999;257:1-6.

10. Bethell DB, Flobbe K, Cao XT, Day NP, Pham TP, Buurman WA, Cardosa MJ, White NJ, Kwiatkowski D. Pathophysiologic and prognostic role of cytokines in dengue hemorrhagic fever. J Infect Dis 1998;177:778-782.

11. Hober D, Nguyen TL, Shen L, Ha DQ, Huong VT, Benyoucef S, Nguyen TH, Bui TM, Loan HK, Le BL, Bouzidi A, De Groote D, Drouet MT, Deubel V, Wattré P. Tumor necrosis factor alpha levels in plasma and whole-blood culture in dengue-infected patients: relationship between virus detection and pre-existing specific antibodies. J Med Virol 1998;54:210-218.

12. Chaturvedi UC, Dhawan R, Mukerjee R. Immuno-suppression and cytotoxicity of dengue infection in the mouse model. In: Dengue and dengue hemorrhagic fever. (Gubler DJ, Kuno G, eds.). CAB International, Wallingford, UK, 1997; pp. 291-311.

13. Huang YH, Lei HY, Liu HS, Lin YS, Liu CC, Yeh TM. Dengue virus infects human endothelial cells and induces IL-6 and IL-8 production. Am J Trop Med Hyg 2000;63:71-75.

14. Waidab W, Suphapeetiporn K, Chalurmpon S, *et al*. Association of cytokine-related gene expression with dengue infection severity. Presented at the 25th International Congress of Pediatrics, August 25-30, 2007, Athens, Greece.

15. Malasit P. Complement and dengue hemorrhagic fever/ shock syndrome. Southeast Asian J Trop Med Public Health 1987;18:316-320.

16. Halstead SB, O'Rourke EJ. Dengue virus and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J Exp Med 1977;146:201-217.

17. Halstead SB, O'Rourke EJ, Allison AC. Dengue viruses and mononuclear phagocytes. II. Identify of blood and tissue leukocytes supporting *in vitro* infection. J Exp Med 1977;146:218-229.

18. Halstead SB. Immunological parameters of togavirus disease syndrome. In: The togaviruses (Schlesinger RW, ed.). Academic Press, New York, USA, 1980; pp. 107-173.

19. Halstead SB. The pathogenesis of dengue: molecular epidemiology in infectious disease. Am J Epidemiol 1981;114:632-648.

20. Green S, Vaughn DW, Kalayanarooj S, Nimmannitya S, Suntayakorn S, Nisalak A, Lew R, Innis BL, Kurane I, Rothman AL, Ennis FA. Early immune activation in acute dengue illness is related to development of plasma leakage and disease severity. J Infect Dis 1999;179:755-762.

21. Leitmeyer KC, Vaughn DW, Watts DM, Salas R, Villalobos I, de Chacon, Ramos C, Rico-Hesse R. Dengue virus structural differences that correlate with pathogenesis. J Virol 1999;73:4738-4747.

22. Murgue B, Roche C, Chungue E, Deparis X. Prospective study of the duration and magnitude of viraemia in children hospitalised during the 1996-1997 dengue-2 outbreak in French Polynesia. J Med Virol 2000;60:432-438.

23. Sudiro TM, Zivny J, Ishiko H, Green S, Vaughn DW, Kalayanarooj S, Nisalak A, Norman JE, Ennis FA, Rothman AL. Analysis of plasma viral RNA levels during acute dengue virus infection using quantitative competitor reverse transcription-polymerase chain reaction. J Med Virol 2001;63:29-34.

24. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA, Nisalak A. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis 2000;181:2-9.

25. Guzmán MG, Kouri G, Valdes L, Bravo J, Alvarez M, Vazques S, Delgado I, Halstead SB. Epidemiologic studies on dengue in Santiago de Cuba, 1977. Am J Epidemiol 2000;152:793-799.

26. Sangkawibha N, Rojanasuphot S, Ahandrik S, Viriyapongse S, Jatanasen S, Salitul V, Phanthumachinda B, Halstead SB. Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. Am J Epidemiol 1984;120:653-669.

27. Rico-Hesse R, Harrison LM, Salas RA, Tovar D, Nisalak A, Ramos C, Boshell J, de Mesa MT, Nogueira RM, da Rosa AT. Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. Virology 1997;230:244-251.

28. Watts DM, Porter KR, Putvatana P, Vasquez B, Calampa C, Hayes CG, Halstead SB. Failure of secondary infection with American genotype dengue 2 to cause dengue haemorrhagic fever. Lancet 1999;354:1431-1434.

29. Guzman MG. Dengue haemorrhagic fever in Cuba, 1981: a retrospective seroepidemiologic study. Am J Trop Med Hygiene 1981;42:179-184.

30. An J, Kimura-Kuroda J, Hirabayashi Y, Yasui K. Development of a novel mouse model for dengue virus infection. Virology 1999;263:70-77.

31. Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 1998;11:480-496.

32. World Health Organization. Dengue haemorrhagic fever: diagnosis, treatment and control, 2nd ed. WHO, Geneva, 1997.

33. Pancharoen C, Thisyakorn U. Dengue virus infection during infancy. Trans R Soc Trop Med Hyg 2001; 95:307-308.

34. Pancharoen C, Thisyakorn U. Dengue infection in teenage children. J Infect Dis Antimicrob Agents 2000;17:93-96.

35. Tantawichien T, Thisyakorn U, Pisarnpong A, Israsena S, Suwangool P. Dengue fever and dengue hemorrhagic fever in adults. The First International Conference on dengue and dengue haemorrhagic fever, Chiangmai, Thailand. November 20-24, 2000. pp. 16-17. (Abstract)

36. Wells RA, Scott RM, Pavanand K, Sathitsathein V, Chiamudom U, Macdermott RP. Kinetics of peripheral blood leukocyte alteration in Thai children with dengue hemorrhagic fever. Infect Immun 1980;28:428-433.

37. Thisyakorn U, Nimmannitya S, Ningsanond V, Soogarun S. Atypical lymphocyte in dengue hemorrhagic fever: its value in diagnosis. Southeast Asian J Trop Med Public Health 1984;15:32-36.

38. Wongsawat J, Pancharoen C, Ningsanond V, Thisyakorn U. Kinetics of peripheral blood leukocyte alterations in Thai children with dengue infection. The First International Conference on dengue and dengue haemorrhagic fever, Chiangmai, Thailand. November 20-24, 2000. pp. 37. (Abstract)

39. Mitrakul C, Thisyakorn U. Hemostatic studies in dengue hemorrhagic fever. In: Proceedings of the 1st International Congress of Tropical Pediatrics; 1989 Nov 8-12; Bangkok, Thailand (Suvatte V, Tuchinda M, eds.). Ruen Kaew Press, Bangkok, Thailand, 1989; pp. 215-217.

40. Murgue B, Cassar O, Guigon M, Chungue E. Dengue virus inhibits human hematopoietic progenitor growth *in vitro*. J Infect Dis 1997;175:1497-1501.

41. Lin CF, Lei HY, Liu CC, Liu HS, Yeh TM, Wang ST, Yang TI, Sheu FC, Kuo CF, Lin YS. Generation of IgM anti-platelet autoantibody in dengue patients. J Med Virol 2001;63:143-149.

42. Bunyavejchevin S, Tanawattanacharoen S, Taechakraichana

N, Thisyakorn U, Tannirandorn Y, Limpaphayom K. Dengue hemorrhagic fever during pregnancy: antepartum, intrapartum and postpartum management. J Obstet Gynaecol Res 1997;23:445-448.

43. Sosothikul D, Seksarn P, Pongsewalak S, Thisyakorn U, Lusher J. Activation of endothelial cells, coagulation and fibrinolysis in children with dengue virus infection. Thromb Haemost 2007;97:627-634.

44. Setrkraising K, Bongsebandhu-phubhakdi C, Voraphani N, Pancharoen C, Thisyakorn U, Thisyakorn C. D-dimer as an indicator of dengue severity. Asian Biomedicine 2007;1:53-57.

45. Scott RM, Nisalak A, Chiamudom U, Seridhoranakul S, Nimmannitya S. Isolation of dengue viruses from peripheral blood leukocytes of patients with hemorrhagic fever. J Infect Dis 1980;141:1-6.

46. Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn S, Puttisri P, Hoke CH. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. Am J Trop Med Hyg 1989;40:418-427.

47. Deubel V, Laille M, Hugnot JP, Chungue E, Guesdon JL, Drouet MT, Bassot S, Chevrier D. Identification of dengue sequences by genomic amplification: rapid diagnosis of dengue virus serotypes in peripheral blood. J Virol Methods 1990;30:41-54.

48. Pupaibool J, Krajiw S, Arunyingmongkol K, *et al.* Diagnosis of dengue infection by enzyme-linked immunosorbent assay and severe transcription polymerase chain reaction from oral specimens. Presented at the 16th European Congress of Clinical Microbiology and Infectious Diseases, April 1-4, 2006, Nice, France.

49. Thisyakorn U, Thisyakorn C. Dengue hemorrhagic fever: Unusual manifestation and problems in management. JAMA SEA 1994;10:102-103.

50. Thisyakorn U, Thisyakorn C. Dengue infection with unusual manifestations. J Med Assoc Thai 1994;77: 410-413.

51. Nimmannitya S, Thisyakorn U, Hemsrichart V. Dengue hemorrhagic fever with unusual manifestations. Southeast Asian J Trop Med Public Health 1978;18:398-406.

52. Thisyakorn U, Thisyakorn C, Limpitikul W, Nisalak A. Dengue infection with central nervous system manifestations. Southeast Asian J Trop Med Public Health 1999;30:504-506.

53. Pancharoen C, Thisyakorn U. Neurological manifestations in dengue patients. Southeast Asian J Trop Med Public Health 2001;32:341-345.

54. Cam BV, Fonsmark L, Hue NB, Phuong NT, Poulsen A, Heegaard ED. Prospective case-control study of encephalopathy in children with dengue hemorrhagic fever. Am J Trop Med Hyg 2001;65:848-851.

55. Solomon T, Dung NM, Vaughn DW, Kneen R, Thao LT, Raengsakulrach B, Loan HT, Day NP, Farrar J, Myint KS, Warrell MJ, James WS, Nisalak A, White NJ. Neurological manifestations of dengue infection. Lancet 2000;355:1053-1059.

56. Pancharoen C, Rungsarannont A, Thisyakorn U. Hepatic function in dengue patients with various severity. J Med Assoc Thailand 2002;85:S298-S301.

57. Innis BL, Myint KSA, Nisalak A. Acute liver failure is one important cause of fatal dengue infection. Southeast Asian J Trop Med Public Health 1990;21:695-696.

58. Pancharoen C, Thisyakorn U. Coinfections in dengue patients. Pediatr Infect Dis J 1998;17:81-82.

59. Tassniyom S, Vasanawathana S, Chirawatkul A, Rojanasuphot S. Failure of high-dose methylprednisolone in established dengue shock syndrome: A placebo-controlled, double-blind study. Pediatrics 1993; 92:111-115.

60. Thisyakorn U. Current status of the knowledge on dengue/dengue hemorrhagic fever/dengue shock syndrome in Thailand. IV. Clinical aspects. Mosquito Borne Diseases Bulletin 1994;11:61-63.

61. Bhamarapravati N, Tuchinda P, Boonyapaknavik V. Pathology of Thailand hemorrhagic fever: a study of 100 autopsy cases. Ann Trop Med Parasitol 1967;61:500-510.

62. Bhamarapravati N, Yoksan S. Live attenuated tetravalent dengue vaccine. Vaccine 2000;18:S44-S47.

63. Kanesa-thasan N, Sun W, Kim-Ahn G, Van Albert S, Putnak JR, King A, Raengsakulsrach B, Christ-Schmidt H, Gilson K, Zahradnik JM, Vaughn DW, Innis BL, Saluzzo JF, Hoke CH Jr. Safety and immunogenicity of attenuated dengue virus vaccines (Aventis Pasteur) in human volunteers. Vaccine 2001;19:3179-3188.

64. Trent DW, Kinney RM, Huang CYH. Recombinant dengue virus vaccines. In: Dengue and Dengue Hemorrhagic Fever (Gubler DJ, Kuno G, eds.). CAB International, Oxon, 1997; pp. 379-403.

65. Kinney RM, Huang CY-H. Development of new vaccines against dengue fever and Japanese encephalitis. Intervirology 2001;44:176-177.

Predicting factors for the experience of HIV testing among women who have given birth in Cambodia

Kazuhiro Kakimoto[1],*, Yuri Sasaki[2], Chushi Kuroiwa[2], Sathiarany Vong[3], Koum Kanal[3]

[1] *International Medical Center of Japan, Tokyo, Japan;*

[2] *The University of Tokyo, Tokyo, Japan;*

[3] *The National Maternal and Child Health Center, Phnom Penh, Cambodia.*

SUMMARY	HIV testing and counselling is an entry point for the prevention of mother-to-child transmission of HIV (PMTCT) services, and it is important to determine predictors for HIV testing to improve the acceptance of HIV testing. The aim of this study was to assess predictive determinants for HIV testing asking mothers who had already given birth. Mothers who came to a childhood immunization in Phnom Penh, Cambodia with a child (children) aged 6-24 months were randomly selected in January and February 2006. A cross-sectional survey with a semi-structured questionnaire including a question about the experience of HIV testing was conducted to the mothers at the clinic by trained interviewers. Of the 315 respondents, 193 mothers (61.3%) had ever been tested for HIV and 265 mothers (84.1%) showed the necessary of permission by partners before HIV testing. In a multivariate logistic regression analysis, basic knowledge about HIV transmission [adjusted odd's ratio (aOR): 2.875, 95% CI: 1.668-4.956] as the best predictor, the number of children (aOR: 2.186, 95% CI: 1.241-3.852) and partner's education level (aOR: 1.950, 95% CI: 1.044-3.641) remained statistically significant, however the necessity of permission by partners did not (aOR: 1.691, 95% CI: 0.859-3.328). Since some mothers ever tested might have obtained the permission with the perception of their partners before tested, it should be still highlighted that involvement of partners is an important strategy. Education on HIV transmission to young women and men through communication and health education strategies involving partners seems to lead PMTCT services to be more acceptable.

Key Words: HIV test, PMTCT, Cambodia, predictors

Introduction

Knowing HIV status through voluntary counselling and testing (VCT) services is essential to preventing new HIV infections and accessing to care, support and treatment for people living with HIV/AIDS. However, the challenges toward the implementation of programmes for the prevention of mother-to-child transmission of HIV (PMTCT) include the low acceptance of HIV testing among pregnant women. The acceptance rates of VCT by pregnant women in PMTCT services varied between 33 and 95% in developing countries (*1*). At antenatal care (ANC), PMTCT services such as counselling and testing are offered pregnant women as part of the services and uptakes of pre-test counselling, HIV testing and post-test counselling affect the efficiency of PMTCT programme implementation. Since the proportion of women living with HIV/AIDS globally continues to grow (*2*), it is important to assess the willingness and the barriers for women to be tested for HIV in order to increase the acceptability of PMTCT services.

Cambodia has the highest HIV prevalence rate in Asia, 1.6% in 2005 (*3*), and approximately 130,000 Cambodian people are estimated to be living with HIV/AIDS whereas fewer than 10,000 are aware of their status (*4*). Although the HIV incidence of sex

workers and policemen successfully decreased from 1999 to 2002 in Cambodia, the incidence of HIV among pregnant women in ANC has not declined (*5*). Under the national policy of the Ministry of Health (*6*), a pilot of the National PMTCT programme using a single dose nevirapine regimen (*7*) commenced with technical and financial support by JICA and UNICEF (*8,9*) in 2001 and the programme has been being scaled up to rural areas by training health personnel such as midwives to be counsellors. The target population of the programme is not only pregnant women living with HIV/AIDS also those who are not infected for the primary prevention. Thus, pre-test counselling, HIV testing and consequent post-test counselling play important roles in the programme. Despite high initial willingness to be tested for HIV, the acceptance of HIV testing among pregnant women was not as high as expected at the beginning of the programme because many women wanted to talk with their partners to obtain the permission by them for being tested before accepting the test (*10*). However, the involvement of their partners in the PMTCT services by inviting them to group health education in ANC and using IEC materials to make them understand more about the services significantly increased the acceptance (*11*). Thus it is necessary to assess whether the permission by their partners was really a barrier and a predictor of decision making to be tested for those who experienced HIV testing and what else could be predictive factors after starting the involvement of their partners.

Most of studies determining barriers and predictors to participate in PMTCT services were conducted on pregnant women at ANC settings in many developing countries. However, no studies carried out on mothers who had already given birth have been reported to assess predictors to receive HIV testing. The aim of this study was to assess the association between the experience of HIV testing and social factors including the permission of their partners for HIV testing, and predictive determinants to be tested by asking mothers who had already given birth.

Materials and Methods

A cross-sectional survey with a semi-structured questionnaire was conducted at a childhood immunization clinic of the National Maternal and Child Health Center (NMCHC) in Phnom Penh, the capital of Cambodia in January and February 2006. Mothers who came to the clinic with a child (children) aged 6-24 months were randomly selected and received information of the study before starting the interview. Before taking part in the study, informed consent was obtained by the interviewers who were trained in the questionnaire and the objectives of the study, and face to face interviews were administered by the interviewers in Khmer language (the most common language in

Cambodia).

Items asked in the questionnaire were experience of HIV testing as a dependent variable, socio-demographic characteristics, necessity of the permission by their male partners for HIV testing and basic knowledge on the transmission of HIV as independent variables. All the items were verbally answered by the respondents. Place of ANC and delivery were asked about their last pregnancy. The knowledge was also measured by asking questions about the possibilities of "HIV transmission via mosquitoes", "mother-to-child transmission of HIV" and "transmission by kissing or hugging". The number of questions each mother answered correctly was totalled to score the knowledge of the mother and those mothers who scored full mark were regarded as having basic knowledge on transmission of HIV.

Data was processed and analyzed in SPSS 11.0 for Windows. Chi square tests were used to compare differences among various independent variables. The relative risks of possible risk factors were estimated by odds ratios (OR) and 95% confidence intervals (CI). Independent variables that had a significant relationship with dependent variables at the $p < 0.05$ level was selected and included in a multivariate logistic regression analysis.

Ethical clearance and approval of the study was obtained from the National Ethic Committee for Health Research in the Ministry of Health, Cambodia before starting the study.

Results

Three hundred and forty three mothers accompanied by their child aged 6-24 months were offered the study and 315 mothers (91.8%) who agreed to participate in it were interviewed. The mean age of the mothers and their youngest children were 27.5 years and 10.7 months, respectively (Table 1). Of the 315 mothers, 314 (99.7%) were married and 150 (47.6%) had one child only. Most of the respondents received ANC and were delivered at health facilities. One hundred and ninety three mothers (61.3%) had ever been tested for HIV and overall the majority of all the respondents thought it is necessary to obtain the permission by their partners for HIV testing.

In a univariate analysis, different variables were examined to determine significant association with the experience of HIV testing. The analysis shows that age of mothers ($p = 0.001$), the number of children ($p < 0.001$), place of ANC ($p = 0.007$), mother's education level ($p = 0.001$), partner's education level ($p < 0.001$), the necessity of partner's permission for HIV testing ($p = 0.016$) and basic knowledge on HIV transmission ($p < 0.001$) were significantly related between the mothers who had been tested and those who had never (Table 2). No statistically significant difference was found for the number of family members ($p = 0.148$) or occupation of

Table 1. Characteristics of the women in the study (*n* = 315)

Average Age (years) (SD)	27.5 (5.3)
Average Age of the youngest child (months) (SD)	10.7 (3.3)
Number of children	
One (%)	150 (47.6)
Two or more (%)	165 (52.4)
Marital status	
Married (%)	314 (99.7)
Single (%)	1 (0.3)
Average of family member (persons) (SD)	5.7 (2.1)
Occupation	
Housewife (%)	190 (60.3)
Government, private company or others (%)	125 (39.7)
Place of ANC	
Health facility (%)	302 (95.9)
Others (%)	13 (4.1)
Place of delivery	
NMCHC (%)	161 (51.1)
Other health facilities	123 (39.0)
Others (%)	31 (9.8)
History of HIV testing	
Ever been tested	193 (61.3)
Never	122 (38.7)
Need of permission by partner	
Yes (%)	265 (84.1)
No or I don't know (%)	50 (15.9)
Basic knowledge on HIV transmission	
Full mark (%)	206 (65.4)
Less (%)	109 (34.6)

Table 3. Multivariate logistic regression analysis of predictors to the experience of HIV testing

	Odd's ratio (95% CI)	*p* value
Age		
More than 27 years old (%)	0.633 (0.360-1.111)	0.111
27 years old and less (%)	1.000	
Number of children		
One (%)	2.186 (1.241-3.852)	0.007
Two or more (%)	1.000	
Place of ANC		
Health facility (%)	3.817 (0.903-16.129)	0.068
Others (%)	1.000	
Necessity of permission by partners		
Yes (%)	1.691 (0.859-3.328)	0.128
No and I don't know (%)	1.000	
Knowledge on HIV transmission		
Full mark (%)	2.875 (1.668-4.956)	< 0.001
Less (%)	1.000	
Education of mothers		
9 years and over (%)	1.367 (0.753-2.483)	0.304
Less than 9 years (%)	1.000	
Education of partners		
9 years and over (%)	1.950 (1.044-3.641)	0.036
Less than 9 years (%)	1.000	

significant.

Discussion

mothers (*p* = 0.738).

In a multivariate logistic regression analysis, only the knowledge, the number of children and partner's education level remained statistically significant (Table 3). The knowledge was shown to be the best predictor of HIV testing [adjusted odd's ratio (aOR): 2.875, 95% CI: 1.668-4.956] and mothers who have only one child were more likely to have accepted HIV testing (aOR: 2.186, 95% CI: 1.241-3.852). Experience of HIV testing increased with high education of partners (aOR: 1.950, 95% CI: 1.044-3.641) as well while the necessity of the permission was not a significant predictor in the multivariate analysis. The odd's ratio of place of ANC in the last pregnancy was the highest but not statistically

Our study targeting women who had given birth showed that the majority (84.1%) of the respondents thought the necessity of the permission by their partners and that age of mothers, the number of children, place of ANC, mother's education, partner's education, necessity of partner's permission for HIV testing and basic knowledge on HIV transmission were significantly associated with the history of HIV testing in the univariate analysis. Similar factors related to acceptance of VCT were previously reported such as education levels of pregnant women (*12,13*), knowledge (*12-15*), confidentiality (*16*), sigma (*17,18*), trust in the health care system (*17*), fear to the response of partners

Table 2. Comparison between mothers who have ever tested and never

	Ever tested (*n* = 193)	Never (*n* = 122)	Odd's ratio (95% CI)	*p* value
Age				
More than 27 years old (%)	60 (31.1%)	60 (49.2%)	0.466	0.001
27 years old and less (%)	133 (68.9%)	62 (50.8%)	(0.292-0.744)	
Number of children				
One (%)	108 (56.0%)	42 (34.4%)	2.420	< 0.001
Two or more (%)	85 (44.0%)	80 (65.6%)	(1.514-3.870)	
Occupation				
Housewife (%)	115 (59.6%)	75 (61.5%)	0.924	0.738
Others (%)	78 (40.4%)	47 (38.5%)	(0.581-1.471)	
Number of family members				
More than 5 (%)	82 (42.5%)	62 (50.8%)	0.715	0.148
5 and less (%)	111 (57.5%)	60 (49.2%)	(0.453-1.127)	
Place of ANC				
Health facility (%)	190 (98.4%)	112 (91.8%)	5.650	0.007*
Others (%)	3 (1.6%)	10 (8.2%)	(1.524-20.833)	
Necessity of permission by partners				
Yes (%)	170 (88.1%)	95 (77.9%)	2.101	0.016
No and I don't know (%)	23 (11.9%)	27 (22.1%)	(1.141-3.867)	
Knowledge on HIV transmission				
Full mark (%)	146 (75.6%)	60 (49.2%)	3.210	< 0.001
Less (%)	47 (24.4%)	62 (50.8%)	(1.979-5.207)	
Education of mothers				
9 years and over (%)	79 (40.9%)	27 (22.1%)	2.438	0.001
Less than 9 years (%)	114 (59.1%)	95 (77.9%)	(1.457-4.080)	
Education of partners				
9 years and over (%)	159 (82.4%)	75 (61.5%)	2.931	< 0.001
Less than 9 years (%)	34 (17.6%)	47 (38.5%)	(1.743-4.928)	

(*19*), and partner involvement such as the permission of partners (*10,12*) and the willingness to disclose test result to the partner (*17*). However, it should be highlighted that the rate of those mothers who showed the necessity of the partner's permission among the tested mothers was significantly higher compared to the rate among the not-tested while this was not anticipated before the study and that the necessity of the permission was not likely to be a determining factor in the multivariate logistic regression model. This result suggests that some mothers with experience of HIV testing in spite of the necessity of the permission probably might have obtained the permission with the perception of their partners about HIV testing and that the necessity of the permission cannot be a barrier to hamper the willingness to be tested in our setting. Accordingly, the finding that the education level of their partners was a predictor in the regression model is reasonable supposing that educated partners are more acceptable to HIV testing of their spouses. A study targeting primary school teachers in Tanzania (*20*) also found that the education level of their partners was significantly more likely to increase the history to be tested for HIV while one third of the samples included male respondents. In addition, the profession of male partners was indicated as the strongest predictor for the acceptability of HIV testing among pregnant women in a report from Rwanda (*21*). Thus, the importance of the partner's involvement in PMTCT services should be nevertheless stressed although the permission by partners was not likely to be a predictor for HIV testing. On the other hand, since the high prevalence of disclosure of HIV status to partners were reported in Cambodia (*11*), the issue of domestic violence by partners (*22*) after disclosure must be paid attention to (*23*) whereas most of the cases were reported in African countries (*24-26*).

In our study, the best independent predictor to the history of HIV testing was the basic knowledge on HIV transmission which was evaluated by asking only three simple questions while other knowledge such as one on HIV testing (*12*) or AIDS treatment was not included in our questions to minimize and simplify the questionnaire. This seems because the HIV testing policy for PMTC in Cambodia adopts "opt-in" strategy. Mothers who have more knowledge could consider what benefit of HIV testing is and accept it. A study assessing the association between health literacy and HIV test acceptance in "opt-out" strategy found that low health literacy may not be a barrier to patients accepting HIV testing when recommended by a health care provider (*27*). Future studies evaluating determining factors of HIV testing experience should include more questions about HIV knowledge to investigate what message is needed and should be promoted through counsellors at health facilities and IEC strategies to increase the acceptability of HIV testing in PMTCT services.

The number of children was also an independent predictive factor of HIV testing in our mothers. The finding that the mothers having one child only increased the uptake of HIV testing agrees with previous studies conducted for pregnant women in African countries (*28,29*). However, it was not clarified whether this was because mothers with two or more children were less likely to be informed on PMTCT or to be interested in HIV testing. PMTCT services in ANC should pay more attention to mothers who have ever given birth as well as primiparae of whom 79.2% want another child either soon or later (*30*).

To our knowledge, this was the first study to investigate predictors to the history of HIV testing among women who have given birth in Cambodia. It can be agreed that an ANC clinic is a good place to ask about willingness of HIV testing as an attitude but not suitable to ask the experience of HIV testing since some mothers might accept the test just before the delivery (*31*) or before the pregnancy. Therefore, the childhood vaccination clinic in Phnom Penh was selected as the study site. It was also considered easier to access women who have given birth, and the study in the childhood vaccination clinic in Phnom Penh could be more generalized to find the situation of the capital of Cambodia than studies conducted in ANC. The coverage of children who were vaccinated against any preventable childhood illnesses in Cambodia was estimated more than 90% (*30,32*), and this was higher than the coverage of ANC attendance in Phnom Penh that was 85% (*30*). Furthermore, Fujita *et al*. (*33*) estimated that approximately 30% of institutional deliveries in Phnom Penh were done in NMCHC while half of the mothers in the study had delivery in NMCHC.

This study, however, had certain limitations, since it was a cross-sectional health facility based study, so we cannot deny the possibility that the mothers interviewed originally had more interest in their own and families' health including HIV testing. In fact, the rate of deliveries at health facilities in our study was higher than other reports. The Cambodia Demographic and Health Survey report in 2005 (CDHS) (*30*) showed that it was 78.9% and Fujita *et al*. (*33*) estimated 84%. Thus, the application of the findings to other population in Phnom Penh still needs careful considerations. In addition, there is no doubt that studies targeting only mothers who came to a childhood vaccination clinic cannot include mothers who had abortion or still birth.

In conclusion, the results showed that the permission by male partners was not a predictor for HIV testing in our setting and that the best predictive factor for HIV testing among mothers who came to a childhood immunisation clinic with their children was basic knowledge on HIV transmission. Promotion of HIV knowledge to young women and men through communication strategies and health education by health care providers involving partners seems to lead HIV testing in PMTCT services to be more acceptable and involve more male partners in the services.

Acknowledgement

References

1. Cartoux M, Meda N, Van de Perre P, Newell ML, de Vincenzi I, Dabis F. Acceptability of voluntary HIV testing by pregnant women in developing countries: an international survey. AIDS 1998;12:2489-2493.
2. UNAIDS/WHO. AIDS Epidemic Update 2006.
3. UNAIDS. Report on the global AIDS epidemic 2006.
4. Bith P. AIDS in Cambodia. Lancet Infect Dis 2004;4:482.
5. Saphonn V, Parekh BS, Dobbs T, Mean C, Bun LH, Ly SP, Heng S, Detels R. Trends of HIV-1 seroincidence among HIV-1 sentinel surveillance groups in Cambodia, 1999-2002. J Acquir Immune Defic Syndr 2005;39:587-592.
6. The ministry of health, the royal government of Cambodia. National Policy on Prevention of Mother-to-Child Transmission of HIV 2000.
7. Guay L, Musoke P, Fleming T, *et al*. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomized trial. Lancet 1999;354:795-802.
8. Kakimoto K, Fujita N. Technical assistance to the national programme for the prevention of mother-to-child transmission in Cambodia. Technology and Development 2004;17:32-39.
9. UNICEF. A UNICEF Fact Sheet 2002: Mother-to-Child Transmission of HIV.
10. Chheng TV, Kakimoto K, Fujita N, Chou TL, Poirot EP, Kanal K. Acceptance of Voluntary Counselling and Testing for Pregnant Women in New National Programme in Cambodia. 15th International AIDS Conference 2002, Barcelona, Spain, abstract D11227.
11. Kakimoto K, Kanal K, Mukoyama Y, Chheng TV, Chou TL, Sedtha C. Influence of the involvement of husbands in the mother class with voluntary confidential counseling and testing acceptance for Prevention of Mother to Child Transmission of HIV Program (PMTCT Program) in Cambodia. AIDS Care 2007;19:381-384.
12. Bajunirwe F, Muzoora M. Barriers to the implementation of programs for the prevention of mother-to-child transmission of HIV: A cross-sectional survey in rural and urban Uganda. AIDS Res Ther 2005;2:10.
13. Lee K, Cheung WT, Kwong VS, Wan WY, Lee SS. Access to appropriate information on HIV is important in maximizing the acceptance of the antenatal HIV antibody test. AIDS Care 2005;17:141-152.
14. Wu Z, Rou K, Xu C, Lou W, Detels R. Acceptability of HIV/AIDS counseling and testing among premarital couples in China. AIDS Educ Prev 2005;17:12-21.
15. Hyodo C, Tanaka T, Kobayashi M, Shimizu M, Prueksunand P, Nitithamyong A, Jittawatanakorn J, Naka S. Factors affecting attitudes towards mother-to-child transmission of HIV among pregnant women in a maternal and child hospital in Thailand. Int J STD AIDS 2000;11:406-409.
16. de Paoli MM, Manongi R, Klepp KI. Factors influencing acceptability of voluntary counselling and HIV-testing among pregnant women in Northern Tanzania. AIDS Care 2004;16:411-425.
17. Castle S. Doubting the existence of AIDS: a barrier to voluntary HIV testing and counselling in urban Mali. Health Policy Plan 2003;18:146-155.
18. Kalichman SC, Simbayi LC. HIV testing attitudes, AIDS stigma, and voluntary HIV counselling and testing in a black township in Cape Town, South Africa. Sex Transm Infect 2003;79:442-447.
19. Maman S, Mbwambo J, Hogan NM, Kilonzo GP, Sweat M. Women's barriers to HIV-1 testing and disclosure: challenges for HIV-1 voluntary counselling and testing. AIDS Care 2001;13:595-603.
20. Kakoko DC, Lugoe WL, Lie GT. Voluntary testing for HIV among a sample of Tanzanian teachers: a search for socio-demographic and socio-psychological correlates. AIDS Care 2006;18:554-560.
21. Kowalczyk J, Jolly P, Karita E, Nibarere JA, Vyankandondera J, Salihu H. Voluntary counseling and testing for HIV among pregnant women presenting in labor in Kigali, Rwanda. J Acquir Immune Defic Syndr 2002;31:408-415.
22. Garcia-Moreno C, Jansen HA, Ellsberg M, Heise L, Watts CH: WHO Multi-country Study on Women's Health and Domestic Violence against Women Study Team. Prevalence of intimate partner violence: findings from the WHO multi-country study on women's health and domestic violence. Lancet 2006;368:1260-1269.
23. Baiden F, Remes P, Baiden R, Williams J, Hodgson A, Boelaert M, Buve A. Voluntary counseling and HIV testing for pregnant women in the Kassena-Nankana district of northern Ghana: is couple counseling the way forward? AIDS Care 2005;17:648-657.
24. Medley A, Garcia-Moreno C, McGill S, Maman S. Rates, barriers and outcomes of HIV serostatus disclosure among women in developing countries: implications for prevention of mother-to-child transmission program. Bull World Health Organ 2004;82:299-307.
25. Maman S, Mbwambo JK, Hogan NM, Weiss E, Kilonzo GP, Sweat MD. High rates and positive outcomes of HIV-serostatus disclosure to sexual partners: reasons for cautious optimism from a voluntary counseling and testing clinic in Dar es Salaam, Tanzania. AIDS Behavior 2003;7:373-382.
26. Temmerman M, Ndinya-Achola J, Ambani J. Piot P. The right not to know HIV-test results. Lancet 1995;345:969-970.
27. Barragan M, Hicks G, Williams MV, Franco-Paredes C, Duffus W, del Ri C. Low health literacy is associated with HIV test acceptance. J Gen Intern Med 2005;20:422-425.
28. Stringer JS, Sinkala M, Stout JP, Goldenberg RL, Acosta EP, Chapman V, Kumwenda-Phiri R, Vermund SH. Comparison of two strategies for administering nevirapine to prevent perinatal HIV transmission in high-prevalence, resource-poor settings. J Acquir Immune Defic Syndr 2003;32:506-513.
29. Pignatelli S, Simpore J, Pietra V, Ouedraogo L, Conombo G, Saleri N, Pizzocolo C, De Iaco G, Tall F, Ouiminga A, Carosi G, Castelli F. Factors predicting uptake of voluntary counselling and testing in a real-life setting in a mother-and-child center in Ouagadougou, Burkina Faso. Trop Med Int Health 2006;11:350-357.
30. National Institute of Public Health, the Ministry of Health Cambodia & National Institute of Statistics, the Ministry of Planning. Cambodia Demographic Health Survey 2005 Preliminary Report 2006.
31. Saman M, Kruy LS, Glaziou P, Rekacewicz C, Leng C, Min DC, Delfraissy JF. Feasibility of antenatal and late HIV testing in pregnant women in Phnom Penh Cambodia: the PERIKAM/ANRS1205 study. AIDS 2002;16:950-951.
32. WHO/UNICEF (2006). Review of National Immunization Coverage 1980-2005.
33. Fujita N, Matsui M, Srey S, Po CS, Uong S, Koum K. Antenatal care in the capital city of Cambodia: Current situation and impact on obstetric outcome. J Obstet Gynaecol Res 2005;31:133-139.

Self-reported health: A study of older adults from a developing country - Nepal

Hom Nath Chalise*, Tami Saito, Ichiro Kai

Department of Social Gerontology, School of Public Health, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

SUMMARY   **Self-reported health is an easy measure of overall health and is useful in identifying persons at risk of a decline in health and the risk of disability in older adults. The purpose of this paper is to identify the correlates of self-reported health for older adults in Nepal. The subjects (N = 137) were members of the Chhetri caste who were 60 years or older (mean age 69.1 ± 7.2 years; 54% women) and living in Katmandu City. Data were collected by face-to-face interviews using three self-reported health questions. A single scale was developed from the three self-reported questions. Bivariate analysis and multiple regression analysis were used to analyze the results. The findings show a correlation between self-reported health and sex, household status, living arrangements, social participation, chronic health problems, functional disability in activities of daily living (ADL), employment status, and economic satisfaction. Results of multiple regression analysis indicate that factors for self-reported health among older adults are chronic health problems, economic satisfaction, functional disability in ADLs, and social participation.**

Key Words: Self-reported health, functional disability, elderly, developing country, Nepal

1. Introduction

Populations in developing countries will be aging rapidly in the coming decades: The number of older persons (those aged 65 or older) in less developed countries is expected to increase from 249 million to 690 million between 2000 and 2030 (*1*). This aging population represents a public health success story, but it simultaneously creates new economic and social challenges (*2*). The elderly are at high risk for disease and disability, and an aging population will place urgent demands on a developing country's health care system that is ill-prepared to handle them (*3*).

The health status of the elderly population in poor countries is not well-known due to a lack of health status indicators. In such situations, self-reported health (SRH) may be an alternative research method to assess the health of the elderly because it is simple, short, and global. Since the 1950s, SRH has been one of the most frequently used variables in gerontological and health research (*4*) in Western and other developed countries. The measurement of SRH is generally ascertained by a single question "In general, would you say your health is …?" that is rated on a five-point Likert scale from excellent to poor.

Past research has shown that self-rated health is a useful proxy for morbidity and mortality patterns in epidemiological studies (*5-7*). Researchers have reported that SRH has independent effects on mortality (*7,8*), new morbidity (*9*), functional ability (*10*), health care utilization and hospitalization (*11*), and recovery from illness (*12*). Highly salient experiential factors--such as physical symptoms, energy level, and limitations in functioning--have had the greatest impact on self-assessment of health (*13,14*). Self-rating has also been shown to be a better estimate of the health status of the elderly than professional ratings carried out by nurses (*15*). Self-rating among older adults has been found to be generally more favorable than physicians' ratings (*16,17*).

Detailed examination of SRH has been largely limited to the developed world. Very few analyses have been published using data from developing countries (*18-20*), largely because of the absence of information

---

*Correspondence to: Department of Social Gerontology, School of Health Sciences and Nursing, University of Tokyo, 7-3-1 Hongo Bunkyo-ku, Tokyo 113-0033, Japan;
e-mail: chalisehkpp@gmail.com

on potentially key determinants of SRH such as acute and chronic morbidity, limitations in activities of daily living (ADLs), and, most importantly, measured physical performance (*21*). International examinations of SRH are particularly valuable because there may be important differences in the association of SRH with other health indicators (*4,18,19*).

Nepal is one of the poorest countries in the world, with more than 40% of its people living below the poverty line, low levels of education (particularly for the elderly), and poor health infrastructure. The overwhelming majority of older individuals live with their children, and mostly their sons (*22*). Alternative sources of financial support outside the family are scarce. In Nepal, research on issues facing older adults using primary data is scarce.

This study thus investigates the factors of SRH in the context of a developing country, Nepal.

2. Methods and Measures

2.1 *Methods*

A face-to-face interview using a structured questionnaire was adopted for the study.

2.1.1 *Study site and study population* Data for this study were taken from a cross-sectional survey of Nepalese elderly in July-August 2005. The study site was Katmandu Metropolitan City, the capital and largest city in Nepal with a population of 671,846 (*23*). For the purpose of this study, an administratively and geographically well-defined ward was selected. The ward was chosen for convenience in terms of accessibility and cooperation. This ward has a total population of 34,488 with 7,848 households (*23*). According to an unpublished source from the Central Bureau of Statistics, there were 1,287 households with at least one older adult of 60 years or older. The sample of this study was derived from a larger study. The sampling process has been reported elsewhere (*24*). A convenient sample of 137 older adults from 1,287 households was chosen based on the following criteria: 1) a responsive older adult from each household 2) who belongs to the Chhetri caste/ethnicity, 3) consents to participation in the study, 4) is able to communicate in the Nepali language, and 5) who lacks apparent cognitive impairment as determined by other family members. Due to their considerable differences, other castes/ethnicities were not included in this study.

The Chhetris are members of the second highest of the four castes ingrained in Nepali society and are traditionally said to be responsible for upholding justice and social harmony. The Chhetris include people in government and military positions. They are predominantly Hindus and are of eastern Indo-Aryan stock. The Chhetris account for the highest proportion

(16%) of Nepal's population.

2.1.2 *Data collection* The interviewers consisted of 10 university students (5 males and 5 females) with training or previous experience working in community health from Tribhuvan University. All interviewers participated in a two-day training session that covered informed consent, ethical issues, and a review of the questionnaire.

2.1.3 *Ethical considerations* The study protocol was approved by the Institutional Review Board of the Graduate School of Medicine of the University of Tokyo in June 2005. The purpose of the study was explained and verbal informed consent was obtained from all participants before interviews. The interviews were conducted in the respondents' homes and averaged an hour. No other family members were present at the time of the interview.

2.2 *Measurements*

2.2.1 *Dependent variable* Like in the previous study by Liang *et al*. (*25*), the dependent variable SRH was assessed in this study via three indicators: (a) a rating of physical health [coded 'very bad' (1) to 'very good' (5)], (b) health in comparison to others one's age [coded 'much worse' (1) to 'much better' (5)], and health compared to last year [coded 'much worse' (1) to 'much better' (5)]. Then the three items were added to form a composite of perceived health. In the present study, internal consistency was maintained with a Cronbach's alpha of 0.76. Results of principal component analysis (see appendices 1 and 2) showed that only one factor with loading greater than 0.7 for all of the variables explained more than 68% of the total variance.

2.2.2 *Independent variables* Functional disability was measured using a scale of 5 activities of daily living (bathing, dressing, using the toilet, transferring from bed-to-chair, and eating) developed from the 6-item ADL scale (*26*). One item, "Using the Telephone," was not included in this scale as it was not practical for the majority of the subjects in this sample. The present study defined ADL disability as a 'needing some assistance'/'inability to do the activity at all' with regard to one or more of the activities listed in the respective scale.

Age was measured as a continuous variable. Marital status was classified as 'married' or 'widow/ widower'. Widow/widower included individuals who were unmarried, divorced, or separated. Household status was classified as 'family member' or 'head of household'. A head of household was defined as an individual who makes major decisions with regard to household activities. Education was classified as 'illiterate' or 'literate'. Total family size was measured

as a continuous variable. Living arrangements in this study were classified as 'living with children' or 'other.' Social participation was measured with the question "Do you participate in social activities (such as political gatherings, local development meetings, or religious work)?" and was answered with 'yes' or 'no.' The existence of chronic health problems also required a 'yes' or 'no.' Currently working for cash or in kind was reported simply as 'yes' or 'no.' Previous occupations were divided into 'agricultural' and 'non-agricultural' employment. Financial satisfaction, a widely used measure of self-perceived financial condition, was assessed by the respondent's estimate of his or her present financial condition on a 5-point Likert scale of 'not satisfied,' 'not that satisfied,' 'fair,' 'satisfied' and 'very satisfied.' Since the distribution at the extremes was small, the present study grouped answers on a 3-point scale with 'not satisfied' and 'not that satisfied' coded as 1, 'fair' coded as 2, and 'satisfied' and 'very satisfied' coded as 3.

3. Analysis

Selected background variables such as age, sex, marital status, household status and education, social participation, chronic health problems, as well as currently working for cash or in kind, previous occupation, functional disability, living arrangements, and self-perception of economic satisfaction and their correlation with the SRH scale were all examined using Spearman's correlation. The variables that significantly correlated ($p < 0.05$) with SRH were then used in multiple regression analysis as potential predictors of SRH. Analysis was conducted using SPSS 14.0 for Windows.

4. Results

The distribution of social, demographic and health-related characteristics of the 137 Chhetri elderly is shown in Table 1. Subjects ranged in age from 60 to 93 years of age with a mean age of 69.1 ± 7.2 years. Seventy-four (54.0%) were women, and seventy (51.1%) were married. Seventy-four (54.0%) were illiterate (meaning they could not read or write). About 66% of the elderly participated in social activities. Only thirty-six (26.3%) were currently working for cash or in kind. Regarding self-reported economic satisfaction, 50% of the elderly reported their situation to be fair. The majority of older adults were living with their children. Eighty-two (59.9%) older adults reported suffering from chronic diseases. The average household size of older adults was 6.0 ± 2.9 members. Eight-point eight percent of the older adults reported having problems with at least one of the ADL items.

Table 2 shows the simple correlation of SRH with selected background variables. Results indicated that being male ($p = 0.035$), head of household ($p = 0.002$), economically satisfied in comparison to one's neighbors ($p = 0.001$), participating in social activities ($p = 0.000$) and being employed ($p = 0.010$) were positively related to SRH. Furthermore, having a self-reported chronic disease ($p = 0.000$), functional disability in ADL ($p = 0.000$), and living with one's children ($p = 0.012$) were negatively correlated with SRH.

The above variables significantly correlating with SRH ($p < 0.050$) were then used in multiple regression analysis. Non-significant variables were then removed from the model.

Results of multiple regression of self-reported health are shown in Table 3. The model summary shows that more than 50% ($R^2 = 0.541$ and adjusted $R^2 = 0.513$) of the variance was explained by 8 variables included in the model: sex, household status, participation in social activities, self-reported chronic health problems, currently working for cash or in kind, economic

Table 1. Selected background characteristics of Chhetri elderly ($N = 137$)

Characteristics	N	%	Mean	SD	Range
Age			69.1	7.2	60-93
Sex (men)	63	46.0			
Marital Status (married)	70	51.1			
Household Status (Head)	82	59.9			
Education (illiterate)	74	54.0			
Total family size			6.0	2.9	1-19
Living arrangement					
With children	118	86.1			
Social participation (yes)	90	65.7			
Chronic health problem (yes)	82	59.9			
Functional disability in ADL* (yes)	12	8.8			
Past occupation					
Non-agriculture	46	33.6			
Working status (Working)	36	26.3			
Economic satisfaction					
Not satisfied	35	25.5			
Fair	69	50.4			
Satisfied	33	24.1			
Self-reported health			8.81	2.01	3-15
General physical health			2.81	0.91	1-5
Compared to other people			2.81	0.71	1-5
Compared to last year			3.21	0.81	1-5

SD = Standard deviation
*Activities of daily living and includes 5 items: bathing, dressing, toileting, transfer, eating.

Table 2. Correlation of background variables with self-reported health

Variables	Spearman's correlation coefficients (r)	p*
Age	0.059	0.496
Sex (men)	0.180	0.035
Maritas Status (married)	0.149	0.083
Household Status (Head)	0.261	0.002
Education (illiterate)	0.097	0.401
Total family size	-0.102	0.237
Living arrangement	-0.213	0.012
Social participation (yes)	0.314	0.000
Chronic health problem (yes)	-0.569	0.000
Functional disability in ADL	-0.318	0.000
Past occupation (Non-agriculture)	0.019	0.378
Working status (Working)	0.220	0.010
Economic satisfaction	0.281	0.001

satisfaction, living arrangements, and functional disability in ADL. The model demonstrated the factors that were related to the self-reported health of the elderly included participation in social activities, self-reported chronic health problems, economic satisfaction compared to one's neighbors, functional disability in ADL and living arrangements. Having chronic health problems (B = -2.026, β = -0.503, t = -8.143, p = 0.000) and functional disability in ADL (B = -1.170, β = -0.168, t = -2.652, p = 0.009) were factors of SRH. On the other hand, social participation (B = 0.663, β = 0.159, t = 2.441, p = 0.016) and self-perceived economic satisfaction (B = 0.760, β = 0.271, t = 4.248, p = 0.000) were positively associated with a better SRH. Living with one's children (B = -0.862, β = -0.157, t = -2.583, p = 0.011) was found to be associated with a lower SRH in older adults.

5. Discussion

In light of increasing worldwide concern for issues affecting the elderly, self-reported health has been recognized as a valid indicator of health status and an important component of the quality of life among older adults. The basic purpose of the present study was to examine the factors of self-reported health among Nepalese (Chhetri) older adults.

The mean score of self-reported health in this study, which used a 3-item self-reported health scale (score ranging from 3-15 points), was 8.77 ± 1.98. This score cannot be compared to scores in other studies since 1-item self-reported health is generally used to study the self-reported health of the elderly. A study by Liang

et al. (*25*) also used a 3-item self-rated scale (two items were similar to items in this study while one differed), but that study did not include the score.

Findings from the current study reveal that self-reported chronic health problems were the most significant variables of self-reported health, mirroring the study by Liang *et al.* (*25*). Further, functional limitation in at least one of the activities of daily living was related to a lower SRH. These findings were also supported by findings from other countries (*27-32*).

Many studies have shown that participation in social activities is good for the mental health of the elderly (*33,34*). The current study also noted that participation in social activities was related to a better SRH. A study by Wu and Hart (*35*) showed that social involvement declines with mobility problems but increases with self-reported health status, whereas social contact also increases with cognitive function. Poor mental health reduces social contact and participation.

Some studies (*36,37*) have indicated that the subjective experience of financial strain is more closely related to health than is the actual level of income. This might be particularly true for older adults. A study from Hong Kong (*38*) found that those who rated their financial situation as insufficient experienced poorer health than those who rated their situation as sufficient. The current findings also demonstrated that self-reported economic satisfaction was related to a better SRH in Nepalese (Chhetri) older adults.

Based on these findings, functional limitations in ADL and having chronic health problems were closely related to a lower SRH; self-reported economic satisfaction and participation in social activities were related to a better SRH among Nepalese older adults. Age, employment status, education, household status, and sex had no effect on self-reported health in the present study. Research from developed and developing countries shows that more health problems are reported by women than men (*18,39-41*). At the bivariate level, men in the current study had a higher self-rated heath, but it did not remain significant in the final model.

Interpretations of this study should be considered within the context of the study's strengths and limitations. First, the data were cross-sectional, so establishing the casuality of correlations between dependent variables and independent variables would

Table 3. Multiple regression of self-reported health*

Variables	Unstandardized coefficient (B)	Standardized coefficent (β)	t	p
Social participation	0.663	0.159	2.441	0.016
Chronic health problem	-2.026	-0.503	-8.143	0.000
Economic satisfaction	0.760	0.271	4.248	0.000
Functional disabilty in ADL	-1.170	-0.168	-2.652	0.009
Living arrangement	-0.862	-0.157	-2.583	0.011
Model Summary				
R^2	0.541			
Adjusted R^2	0.513			

*Only significant variables are shown from the variables entered: sex, household status, living arrangement, social participation, chronic health problem, activities of daily living, working status and self-perceived economic.

be difficult. Therefore, future research needs to test the causality between independent variables and SRH of the elderly using a longitudinal design. Second, the current results cannot be extrapolated to the distribution of self-rated health in other populations in Nepal in light of the convenient nature of the sample. Because the present study is a correlation study, however, results indicating correlations between variables may be valid in other populations. Further study is necessary to confirm the current findings in other settings.

Despite these limitations, this study should prove useful for examination of aging populations in developing countries and improvement of gerontological care and future health policy in Nepal since it is the first to analyze the significant variables of SRH among Nepalese elderly. A better understanding of the factors influencing self-reported health will help in the development of preventive interventions to maintain and improve the quality of life of older adults.

Acknowledgments

References

1   Kinsella K, Velkoff VA. An Aging World: 2001 (Washington, DC: U.S. Census Bureau).
2   Hambleton I, Clarke K, Broome H, Fraser H, Brathwaite F, Hennis A. Historical and current predictors of self-reported health status among elderly persons in Barbados. Rev Panam Salud Publica 2005;17:342-352.
3   Kaneda T. Health Care Challenges for Developing Countries with Aging Populations. Population Reference Bureau: 2006.
4   Jylhä M, Guralnik JM, Ferrucci L, Jokela J, Heikkinen E. Is self-rated health comparable across cultures and genders? J Gerontol Soc Sci 1998;53:S144-S152.
5   Subramanian SV, Kawachi I, Kennedy BP. Does the state you live in make a difference? Multilevel analysis of self-rated health in the US. Soc Sci Med 2001;53:9-19.
6   Kawachi I, Kennedy BP, Glass R. Social capital and self-rated health: A contextual analysis. Am J Public Health 1999;89:1187-1193.
7   Idler EL, Benyamini Y. Self-rated health and mortality: A review of twenty-seven community studies. J Health Soc Behav 1997;38:21-37.
8   Benyamini Y, Idler EL. Community studies reporting association between self-rated health and mortality: Additional studies, 1995-1998. Res Aging 1999;21:392-401.
9   Møller L, Kristensen TS, Hollnagel H. Self rated health as a predictor of coronary heart disease in Copenhagen, Denmark. J Epidemiol Community Health 1996;50:423-428.
10 Idler EL, Kasl S. Self-ratings of health: Do they also predict change in functional ability? J Gerontol Soc Sci 1995;50:S344-S353.
11 Mutran E, Ferraro KF. Medical need and use of services among older men and women. J Gerontol Soc Sci 1988;43:S162-S171.
12 Wilcox V, Kasl S, Idler EL. Self-rated health and physical disability in elderly survivors of a major medical event. J Gerontol Soc Sci 1996;51:S96-S103.
13 Benyamini Y, Leventhal H, Leventhal EA. Self-assessments of health: What do people know that predicts their mortality? Res Aging 1999;21:477-500.
14 Benyamini Y, Leventhal H, Leventhal EA. Self-rated oral health as an independent predictor of self-rated general health, self-esteem and life satisfaction. Soc Sci Med 2004;59:1109-1116.
15 Thorslund M, Norström T. The relationship between different survey measures of health in an elderly population. J Appl Gerontol 1993;12:61-70.
16 LaRue A, Bank L, Jarvik L, Hetland M. Health in old age: how do physicians' ratings and self-ratings compare? J Gerontol 1979;34:687-691.
17 Ferraro KF. Self-ratings of health among the old and the old-old. J Health Soc Behav 1980;21:377-383.
18 Rahman MO, Strauss J, Gertler P, Ashley D, Fox K. Gender differences in adult health: An international comparison. The Gerontologist 1994;34:463-469.
19 Zimmer Z, Natividad J, Lin H, Chayovan N. A cross-national examination of the determinants of self-assessed health. J Health Soc Behav 2000;41:465-481.
20 Ahmad K, Jafar TH, Chaturvedi N. Self-rated health in Pakistan: results of a national health survey. BMC Public Health 2005;5:51.
21 Rahman MO, Barsky AJ. Self-reported health among older Bangladeshis: How good a health indicator is it? Gerontologist 2003;43:856-863.
22 Chalise HN, Brightman JD. Aging trends: Population aging in Nepal. Geriatr Gerontol Int 2006;6:199-204.
23 Central Bureau of Statistics (CBS). Population monograph of Nepal: 2003. Volume 1, Katmandu.
24 Chalise HN, Saito T, Takahashi M, Kai I. Relationship specialization amongst sources and receivers of social support and its correlations with loneliness and subjective well-being: A cross-sectional study of Nepalese older adults. Arch Gerontol Geriatr 2007;44:299-314.
25 Liang J, Shaw BA, Krause N, Bennett JM, Kobayashi E, Fukaya T, Sugihara Y. How does self-assessed health change with age? A study of older adults in Japan. J Gerontol B Psychol Sci Soc Sci 2005;60:S224-S232.
26 Katz S, Ford AB, Moskowitz RW, Jackson BA, Jaffe MW. The index of ADL: A standardized measure of biological and psychological function. JAMA 1963;185:914-919.
27 Bryant LL, Beck A, Fairclough DL. Factors that contribute to positive perceived health in an older population. J Aging Health 2000;12:169-192.
28 Leinonen R, Heikkinen E, Jylhä M. Predictors of decline in self-assessments of health among older people--a 5-year longitudinal study. Soc Sci Med 2001;52:1329-1341.
29 Lee Y, Shinkai S. A comparison of correlates of self-rated health and functional disability of older persons in the Far East: Japan and Korea. Arch Gerontol Geriatr 2003;37:63-76.
30 Christensen H, Jorm AF, Henderson AS, Mackinnon AJ, Korten AE, Scott LR. The relationship between health and cognitive functioning in a sample of elderly people in the community. Age Ageing 1994;23:204-212.
31 Heikkinen E, Leinonen R, Berg S, Schroll M, Steen B. Levels and associates of self-rated health among 75-year-old people living in three Nordic localities. In: Functional status, health and aging: the NORA study.

Facts, research and intervention in geriatrics (Heikkinen E, Berg S, Schroll M, Steen B, Viidik A, eds.). Serdi Publisher, Paris, 1997; pp. 121-148.

32 Leinonen R, Kaprio J, Jylhä M, Tolvanen A, Koskenvuo M, Heikkinen E, Rantanen T. Genetic influences underlying self-rated health in older female twins. J Am Geriatr Soc 2005;53:1002-1007.

33 Aquino JA, Russell DW, Cutrona CE, Altmaier EM. Employment status, social support, and life satisfaction among the elderly. J Couns Psychol 1996;43:480-489.

34 Caro FG, Bass SA. Receptivity to volunteering in the immediate postretirement period. J Appl Gerontol 1997;16:427-441.

35 Wu Z, Hart R. Social and health factors associated with support among elderly immigrants in Canada. Res Aging 2002;24:391-412.

36 Ullah P. The association between income financial strain and psychological well-being among unemployed youths. J Occup Psychol 1990;63:317-330.

37 Wilkinson RG. Unhealthy societies: The afflictions of inequality, Routledge, London, 1996.

38 Cheng YH, Chi I, Boey KW, Ko LS, Chou KL. Self-rated economic condition and the health of elderly persons in Hong Kong. Soc Sci Med 2002;55:1415-1424.

39 Wolinsky FD, Johnson RJ. Perceived health status and mortality among older men and women. J Gerontol B Psychol Sci Soc Sci 1992;47:S304-S312.

40 Kumar VK, Sivan YS, Das RRR, Kutty VR. Health of the elderly in a community in transition: a survey in Thiruvananthapuram City, Kerala, India. Health Policy Plan 1994;9:331-336.

41 Kabir ZN, Tishelman C, Torres-Aguero H, Chowdhury AMR, Windblad B, Hojer B. Gender and rural-urban differences in reported health status by older people in Bangladesh. Arch Gerontol Geriatr 2003;37:77-91.

Appendix 1: Matrix

SRH	Component 1
1. General physical health	0.851
2. Compared to others one's age	0.873
3. Compared to last year	0.745

Extraction method: Principal component analysis

Appendix 2: Total variance explained

Component	Initial eigenvalues		Extraction sums of squared loadings	
	Total	% of Variance	Total	% of Variance
1	2.041	68.048	2.041	68.048
2	0.614	20.47		
3	0.344	11.482		

Extraction method: Principal component analysis

Construction of a recombinant single chain antibody recognizing nonreducing terminal mannose residues for use in immuno-histochemistry

Noriyuki Yuasa[1, 2], Noriko Iida[1, 2], Hiroyuki Sakaue[1, 2], Wei Zhang[1, 2], Sharon Wilczynski[3], Yoko Fujita-Yamaguchi[1, 2, 4], *

[1] *Department of Applied Biochemistry, Tokai University School of Engineering, Hiratsuka, Kanagawa, Japan;*

[2] *Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Japan;*

[3] *Department of Anatomic Pathology, City of Hope Medical Center, Duarte, CA, USA;*

[4] *Division of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, CA, USA.*

SUMMARY **We recently reported characterization of 25 clones isolated from a phage library displaying human scFvs using a neoglycolipid, Man3-DPPE, that was synthesized from mannotriose (Man3) and dipalmitoylphosphatidylethanolamine (DPPE). Of those, 5A3 scFv was successfully expressed and purified as a humanized scFv-Fc form (Sakai *et al.,* Biochemistry 46:253, 2007, Zhang *et al. ibid* 263). To carry out immunohistochemistry (IHC) in human tissues, an HA tag sequence was introduced to the 5A3 scFv-Fc gene and the resulting construct was transfected to murine myeloma NS0 cells. The 5A3 scFv-Fc-HA protein expressed was affinity-purified. Sodium dodecyl sulfate polyacrylamide gel electrophoresis under nonreducing and reducing conditions and enzyme-linked immunosorbent assay confirmed that 5A3 scFv-Fc-HA protein is dimeric and retained the ability to recognize nonreducing terminal mannose residues. IHC staining of non-neoplastic tissues by this recombinant antibody revealed that immunoreactivity was not detected in most of the 16 tissues examined. Exceptions were found in IHC staining of the kidney and pancreas, which demonstrated clear staining of proximal tubules and islets of Langerhans, respectively. These results demonstrated that nonreducing terminal mannose residues are not usually present under normal physiological conditions. This study thus provides a potentially useful tool for examination of nonreducing terminal mannose residues that may become apparent under certain pathophysiological conditions.**

Key Words: scFv-Fc, recombinant antibody, high mannose-specific antibody, IHC, human tissue

Introduction

Since many carbohydrates are self-antigens by nature, an *in vitro* approach such as phage display technology is the best strategy to produce anti-carbohydrate antibodies. The authors recently reported characterization of 25 clones isolated from a phage library displaying human scFvs using a neoglycolipid, Man3-DPPE, that was synthesized from mannotriose (Man3) and dipalmitoylphosphatidylethanolamine (DPPE) (*1*). Of those, 5A3 scFv was successfully expressed and purified as a humanized scFv-Fc form (*2*). During the previous study in which several anti-Man3 scFv-Fc constructs were to be expressed in mammalian cells, most transfected clones did not grow nor survive (*2*), which strongly suggests that antibodies against the Man3-moiety are toxic to mammalian cells. Since Man3 is the core carbohydrate for *N*-glycans, which are essential in many glycoproteins with critical biological

functions, inhibition of *N*-glycan synthesis by anti-Man3 antibodies could conceivably have a detrimental impact on cell function (*3-5*). Thus, production of antibodies against Man3 epitopes, nonreducing terminal mannose residues in particular, *in vivo* is presumably not possible. Furthermore, such Man3 epitopes are probably not detectable in tissues under normal physiological conditions since nonreducing terminal mannose residues are masked by other carbohydrates such as *N*-acetyl glucose, galactose, and sialic acids when *N*-glycan synthesis is completed. 5A3 scFv-Fc is most likely the only antibody available for evaluation of tissue expression of such Man3 epitopes. To carry out IHC, a HA tag was introduced to 5A3 scFv-Fc so that its specific binding to the epitopes in human tissues could be clearly detected. This study describes construction of a 5A3 scFv-Fc-HA recognizing nonreducing terminal mannose residues for use in IHC.

Materials and Methods

Materials

The cDNA clone of human IgG1 Fc, composed of hinge, CH2, and CH3, was originally from Dr. J. Schlom, Laboratory of Tumor Immunology and Biology, Division of Cancer Biology and Diagnosis, NCI (Bethesda, MD, USA). Plasmid pEE12.4 and a murine myeloma NS0 cell strain were obtained from Lonza Biologics (Slough, UK). The expression vector SFpEE plasmid was as described in previous reports (*2*). EndoFree Plasmid Maxi (Cat. No. 12362) was purchased from QIAGEN Inc. (Valencia, CA, USA). Dulbecco's modified Eagle medium (DMEM, high glucose) was purchased from Invitrogen (Paisley, UK). Goat anti-human IgG (Fc fragment specific) and peroxidase-conjugated rabbit anti-human IgG secondary antibody were from Jackson Immunoresearch Inc. (West Grove, PA, USA). Peroxidase-conjugated rabbit anti-HA antibody was purchased from BETHYL Laboratories, Inc. (Montgomery, TX, USA). A DAB detection kit was purchased from Invitrogen. Protein A-Sepharose was obtained from Bio-Rad (Hercules, CA, USA). Man3-BSA was obtained from Dextra Laboratories (Reading, UK).

Bacterial strains and transformation

Escherichia coli strain JM109 (TaKaRa Shuzo, Kyoto, Japan) was used as a host for all plasmid preparations Plasmid transformations were performed by electroporation using the Gene Pulser Xcell™ Apparatus (Bio-Rad) under conditions of capacitance at 25 μF, a resistor at 200 ohms (pulse controller), and voltage at 1.5 kV. Resulting bacteria were cultured in LB medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, pH 7.2) containing ampicillin (100 mg/L).

*Modification of vector for expression of HA-tag fusion scFv-Fc*

HA-tag (YPYDVPDYA) was chosen to be added to the C-terminal of scFv-Fc since this epitope tag is known to be recognized by several commercially available antibodies. Human IgG1 Fc cDNA was amplified from the plasmid SFpEE (*2*) using the forward primer 5' -TGG CGC GCC ATT AAT TAA G-3' and the reverse primer 5'-CCG GAA TTC TCA TCA AGC GTA ATC TGG AAC ATC GTA TGG GTA TTT CCC GGG AGA CAG GGA GA-3' to introduce *Asc*I and *Eco*RI restriction sites (solid underline) and HA-tag coding sequence (dotted underline), respectively, and inserted into the *Asc*I/*Eco*RI linearized expression vector SFpEE. The modified expression vector, designated pSF-HA (Figure 1), was identified by restriction enzyme digestions and verified by DNA sequencing.

Vector construction for expression of 5A3 scFv-Fc-HA

To construct an expression vector, the 5A3 scFv gene prepared by digesting SFpEE/5A3 with AscI and PacI was subcloned into *Asc*I-*Pac*I-digested pSF-HA to generate pSF-HA/5A3 (Figure 1). The sequences of resulting vectors were confirmed by DNA sequencing. The expression vector used for electroporation into mammalian cells was purified by QIAGEN EndoFree Plasmid Maxi and stored in sterile water.

Cell culture, transfection and screening

The murine myeloma NS0 cell line was grown in
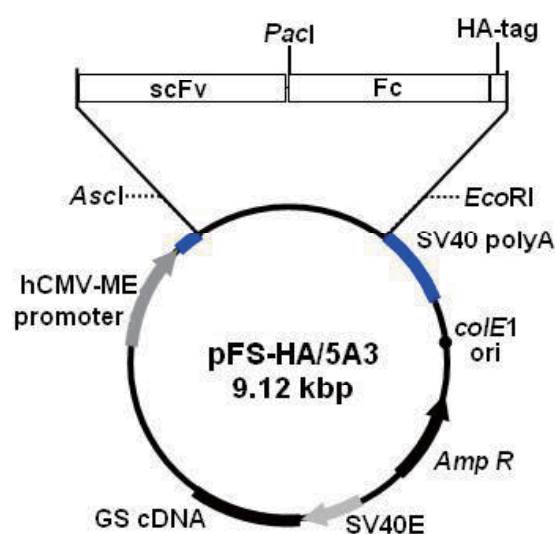
Figure 1. Schematic drawing of the pSF-HA and pSF-HA/5A3 plasmid. GS cDNA, Glutamine synthetase gene; Amp R, Ampicillin resistance gene. The 5A3-scFv gene digested from the plasmid SfpEE/5A3 was inserted into pSF-HA under the control of hCMV-ME promoter as an AscI/PacI fragment. The expressed 5A3 scFv-Fc-HA was expected to be a fusion protein of 491 amino acids with a molecular weight of 54421 that contains a HA-tag at the C-terminus for detection.

DMEM supplemented with 10% FBS and 2 mM glutamine. Selective medium for human Fc-HA-expressing NS0 cells consisted of glutamine-free DMEM, dialyzed FBS, and glutamine synthetase supplement (JRH Biosciences, Lenexa, KS, USA). In a 0.4 cm cuvette, 40 µg of linearized DNA was transfected into 1×10^7 NS0 cells under conditions of 250 volts and 400 µF capacitance. Cells at 1.6×10^5 cell/mL (50 µL/well) were plated in 96-well plates in nonselective media. Twenty-four hours post-electroporation, 150 µL of selective medium were added to each well, and cells were allowed to recover and grow undisturbed for about 3 weeks until discrete surviving colonies appeared.

Supernatants of transfected cells that were able to grow under selective conditions were screened for scFv-Fc-HA protein secretion by sandwich ELISA. Goat anti-human IgG (Fc fragment specific) was diluted in PBS to a final concentration of 10 µg/mL and coated onto 96-well plates, by plating 100 µL/well and incubating at 4°C overnight. After blocking with 3% BSA in PBS, cell culture supernatants from different clones were added and incubated at 37°C for 1 h. After the samples were washed with PBS, horse radish peroxidase (HRP)-conjugated anti-human IgG antibody diluted to 1:20,000 was added to the wells. The wells were washed with PBS and then incubated with 2,2'-azino bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS). The color produced in the wells of plates was analyzed spectrophotometrically at 415 nm on a microplate reader (Bio-Rad model 680).

Purification of scFv-Fc-HA antibody

One of the clones, A4G12 for 5A3 scFv-Fc-HA, was cultured on a large scale in T175 flasks in selective medium. After culturing for 24-48 h, cell culture supernatants were collected and adjusted to pH 8.0 by adding 1/20 volume 1.0 M Tris (pH 8.0) and then applied to a protein A column (5 mL). The column was washed with 10 column volumes of 100 mM Tris-HCl buffer, pH 8.0. The column was then washed with 100 mM citrate buffer, pH 4.5, to remove bovine IgG derived from FBS (*6*). scFv-Fc-HA was eluted from the column with 100 mM citrate buffer, pH 3.0 (*7*). The eluates were collected in 1.5-mL conical tubes containing 1/10 volume 1 M Tris-HCl, pH 8.0. The purified 5A3 scFv-Fc-HA was dialyzed against PBS and concentrated with Amicon® Ultra (Millipore, Billerica, MA, USA). Protein concentration was measured by BCA assay (Pierce, Rockford, IL, USA) with BSA as a standard according to the manufacturer's protocol.

SDS-PAGE

The purified 5A3 scFv-Fc-HA was analyzed by SDS-PAGE, followed by staining with Coomassie Brilliant Blue (CBB) R-250.

ELISA

Solid-phase ELISA was used to confirm the antigen-binding activity of purified 5A3 scFv-Fc-HA against Man3-BSA. Briefly, the antigen, Man3-BSA was diluted to 5 µg/mL in PBS, and coated onto 96-well ELISA plates (100 µL/well) at 4°C overnight with triplicate samples. After blocking of the sample with 3% BSA/PBS, 5A3 scFv-Fc-HA was added. After 2 h of incubation at room temperature, the bound scFv-Fc-HA was detected with HRP-conjugated rabbit-anti HA antibody (1:25,000 dilution) using ABTS as a substrate. After 15 min of incubation in the dark, the reaction was terminated by adding 100 µL of 2% oxalic acid solution. Absorbance at 415 nm was measured using a plate reader.

Immunohistochemistry

Multi-tumor blocks of human tissues were derived from formalin-fixed, paraffin-embedded archives that had been prepared and stored by the Department of Anatomic Pathology, City of Hope Medical Center (*8*). The study was approved by the IRB of the City of Hope Medical Center. Paraffin sections (4 µm thick) mounted on Probe-on slides (Biotek Solutions; Tucson, AZ, USA) were baked for 1 h at 55°C and then dried overnight at 48°C. They were subsequently deparaffinized in xylene, rehydrated in graduated alcohols to distilled water, and loaded into a Techmate slide holder and placed into 10 mM citrate buffer, pH 6.0. Antigen retrieval was performed by the heat-induced epitope retrieval method (*9*). The slides were steamed in 10 mM citrate buffer for 20 min using a household Black and Decker Steamer (model no. HS90) and then allowed to cool for 5 min. The primary scFv-Fc-HA antibody was used at a concentration of 0.14 mg/mL and incubated overnight in a humid container. The scFv-Fc-HA protein was visualized by reaction with HRP-conjugated rabbit-anti HA antibody (1:300 dilution) followed by a modified ABC technique (Vector Elite Kit; Vector Lab, Burlingame, CA, USA). The slides were lightly counterstained with Mayer's hematoxylin.

Results and Discussion

Production and purification of scFv-Fc-HA protein

The constructed 5A3 scFv-Fc-HA gene was introduced into NS0 cells to produce scFv-Fc-HA protein using the GS/NS0 cell system as previously described (*2*). Of 24 stable clones grown in selective media, Fc-positivity was detected by ELISA in the media of 16 clones (data not shown). The scFv-Fc-HA protein (390 µg) was purified from 240 mL culture medium of an A4G12 clones by protein A-column chromatography. The level of 5A3 scFv-Fc-HA protein expression in the medium
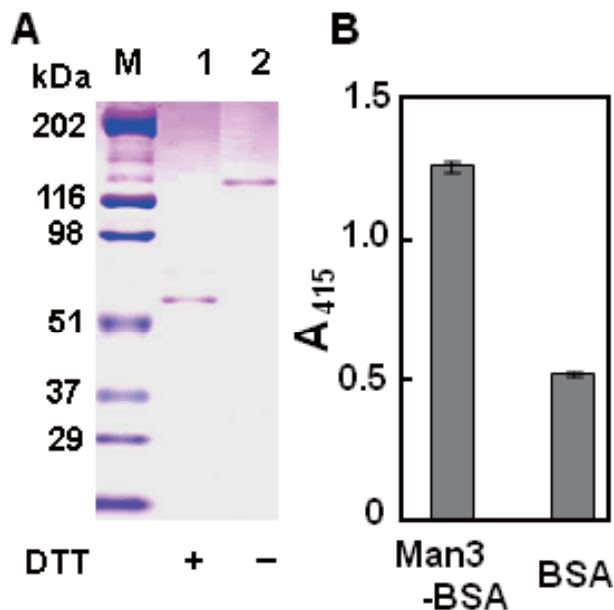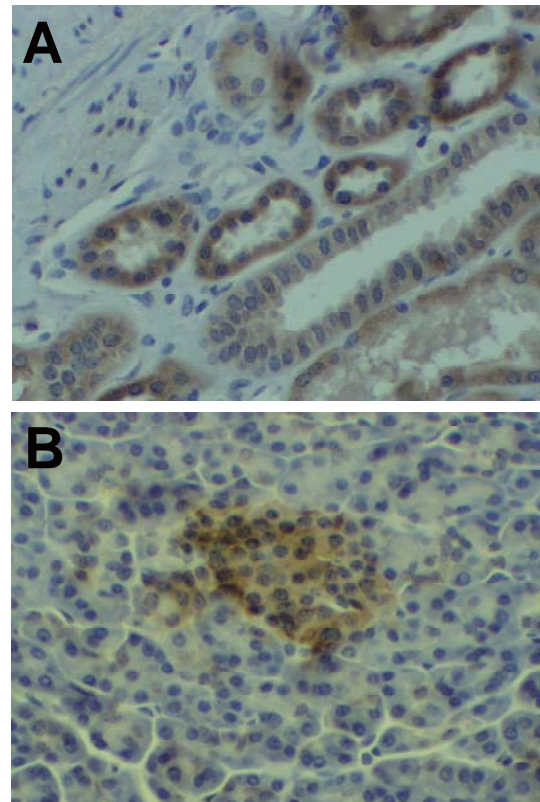
Figure 2. SDS-PAGE and ELISA analyses of the affinity purified 5A3 scFv-Fc-HA protein. (A) CBB staining of a 4-20% acrylamide gel showing 5A3 scFv-Fc-HA under reducing conditions (Lane 1) or under non-reducing conditions (Lane 2), and molecular weight markers (M). (B) Binding of 5A3 scFv-Fc-HA protein to Man3 conjugated with BSA as measure by ELISA. BSA conjugate (0.5 μg/well) was absorbed onto the surface of wells. The purified 5A3 scFv-Fc-HA (3.5 μg/well) was applied to the wells, and the bound 5A3 scFv-Fc-HA was detected with HRP conjugated anti-HA antibody.

Figure 3. IHC staining for Man3 by the recombinant single chain antibody recognizing nonreducing terminal mannose residues, 5A3 scFv-Fc-HA, in non-neoplastic tissues. A, kidney; B, pancreas.

was thus calculated to be approximately 1.63 μg/mL.

Purity and binding activity of purified 5A3 scFv-Fc-HA protein

The purified 5A3 scFv-Fc-HA protein was analyzed by SDS-PAGE under reducing and nonreducing conditions (Figure 2A lanes 1 and 2, respectively). Since human IgG1 Fc contains a hinge region, the scFv-Fc-HA produced by mammalian cells is expected to form a disulfide-linked dimer. The results clearly show that 5A3 scFv-Fc-HA protein is a dimer consisting of two monomers. The molecular mass of the monomeric 5A3 scFv-Fc-HA as estimated by SDS-PAGE was 55-57 kDa, which is consistent with an expected molecular mass of 54 kDa from an amino acid sequence with a contribution of *N*-glycation.

The 5A3 scFv-Fc-HA is thus a divalent recombinant antibody that has an expected molecular mass of 110.4 kDa. The molecular mass of the dimeric scFv-Fc under nonreducing conditions estimated by SDS-PAGE was not exactly double that of the monomer. This is possibly due to post-translational modifications with *N*-glycation in the Fc regions as well as aberrant mobility caused by a rather high polyacrylamide gel concentration for the dimmer size. Therefore, one can conclude that the S-S bonds between two 5A3 scFv-Fc-HA monomers were correctly formed as expected based on its amino acid

sequence.

ELISA analysis of 5A3 scFv-Fc-HA protein against the Man3 antigen

To confirm that the addition of a HA-tag with nine amino acids to the C-terminal of 5A3 scFv-Fc did not disturb its binding activity, the purified 5A3 scFv-Fc-HA protein was subjected to ELISA analysis. The binding of 5A3 scFv-Fc-HA protein to Man3-BSA coated onto plastic plates is shown in Figure 2B. The results confirmed that the purified 5A3 scFv-Fc-HA retained the ability to recognize the Man3 epitope, suggesting that it is suitable for IHC.

IHC staining for nonreducing terminal mannose residues in non-neoplastic tissues by 5A3 scFv-Fc-HA

Multi-tumor blocks of 16 different human tissues containing a total of 70 specimens were examined for immunoreactivity with 5A3 scFv-Fc-HA protein. IHC staining of non-neoplastic tissues carried out with the purified 5A3 scFv-Fc-HA basically revealed no immunoreactivity except that IHC of the kidney and pancreas demonstrated clear staining of proximal tubules (Figure 3A) and islets of Langerhans (Figure 3B), respectively. No immunoreactivity was found in tissue sections including the spleen, uterus, prostate,

ovaries, skin, colon, duodenum, and brain whereas some possible positivity was found in the lung, liver, and testes; this finding requires further examination. These results are consistent with the original prediction that Man3 epitopes are not detectable in tissues under normal physiological conditions as long as *N*-glycation is complete.

In summary, a HA tag was successfully introduced to 5A3 scFv-Fc gene to produce a 5A3 scFv-Fc-HA protein. The purified 5A3 scFv-Fc-HA was dimeric and retained its ability to recognize nonreducing terminal mannose residues. IHC staining for Man3 epitopes in non-neoplastic tissues revealed that immunoreactivity was not detectable in most of the tissues examined, which confirmed that nonreducing terminal mannose residues are not present under normal physiological conditions as predicted. This study thus provided a potentially useful tool for examination of the presence of nonreducing terminal mannose residues that may become apparent under certain pathophysiological conditions. In fact, preliminary IHC studies with 5A3 scFv-Fc-HA detected aberrant expression of nonreducing terminal mannose residues in several cancers. Further investigation is underway.

Acknowledgements

References

1. Sakai K, Shimizu Y, Chiba T, Matsumoto-Takasaki A, Kusada Y, Zhang W, Nakata M, Kojima N, Toma K, Takayanagi A, Shimizu N, Fujita-Yamaguchi Y. Isolation and characterization of phage-displayed single chain antibodies recognizing nonreducing terminal mannose residues. 1. A new strategy for generation of anti-carbohydrate antibodies. Biochemistry 2007;46:253-262.
2. Zhang W, Matsumoto-Takasaki A, Kusada Y, Sakaue H, Sakai K, Nakata M, Fujita-Yamaguchi Y. Isolation and characterization of phage-displayed single chain antibodies recognizing nonreducing terminal mannose residues. 2. Expression, purification, and characterization of recombinant single chain antibodies. Biochemistry 2007;46:263-270.
3. Kukuruzinska MA, Lennon K. Protein *N*-glycosylation: molecular genetics and functional significance. Crit Rev Oral Biol Med 1998;9:415-448.
4. Dennis JW, Granovsky M, Warren CE. Glycoprotein glycosylation and cancer progression. Biochim Biophys Acta 1999;473:21-34.
5. Dennis JW, Granovsky M, Warren CE. Protein glycosylation in development and disease. Bioessays 1999;21:412-421.
6. Schmerr MJ, Patterson JM, Van der Maaten MJ, Miller JM. Conditions for binding bovine IgG1 to protein A-Sepharose. Mol Immunol 1985;22:613-616.
7. Duhamel RC, Schur PH, Brendel K, Meezan E. pH gradient elution of human IgG1, IgG2 and IgG4 from protein A-sepharose. J Immunol Methods 1979;31: 211-217.
8. Battifora H. The multitumor (sausage) tissue block: novel method for immunohistochemical antibody testing. Lab Invest 1986;55:244-248.
9. Baserga R. The insulin-like growth factor I receptor: a key to tumor growth? Cancer Res 1995;55:249-252.

Inflammatory pseudotumor of the spleen: clinical impact in surgical treatment

Koji Takamoto[1], Yutaka Midorikawa[1, 2], *, Masami Minagawa[1], Masatoshi Makuuchi[1]

[1] *Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, the University of Tokyo, Tokyo, Japan;*

[2] *Teikyo University School of Medicine, Mizonokuchi Hospital, Kanagawa, Japan.*

SUMMARY	An inflammatory pseudotumor of the spleen is a rare benign tumor and designated as mass-like lesions with histologic features of nonspecific inflammation and mesenchymal repair although its etiopathogenesis still remains unknown. Here we describe the case of an inflammatory pseudotumor of the spleen in a 57-year-old woman, whose lesion was accidentally found and thought to be lymphoma at first. Generally splenic tumors are difficult to diagnose exactly before surgery, then the patient underwent splenectomy, followed by histopathological diagnosis of inflammatory pseudotumor of the spleen. The optimal management of the asymptomastic patient with such disease is still controversial. The clinical and pathological features of previously reported cases are also reviewed in this paper.

Key Words: Inflammatory pseudotumor, splenic tumor

Introduction

Inflammatory pseudotumors (IPTs) are benign entities of unknown etiology and pathogenesis (*1*). They have been observed in various parts of the body, including the orbit, respiratory tract, gastrointestinal tract and soft tissues, lymph nodes and liver (*2,3*). However, an IPT of the spleen is extremely rare and are frequently misdiagnosed as malignant neoplasms or other benign tumors (*4*). To our knowledge, since Cotelingam and Jaffe first reported 2 cases of splenic IPT in 1984, only 76 cases had been reported in the literature till now (*5*). Although recent advances in imaging techniques, such as ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI), have aided in the identification of space-occupying lesions of the spleen, these techniques do not permit preoperative diagnosis of these lesions (*6*).

In this report, we report an IPT of the spleen and pay particular attention to the incidence, differential diagnosis and treatment of such tumors.


**Correspondence to:* Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, the University of Tokyo, 7-3-1 Hongo, Buknyo-ku, 113-8655 Tokyo, Japan; e-mail: mido-tky@umin.ac.jp

Case Report

A 57-year-old woman admitted to our hospital, pointed out a splenic tumor incidentally by abdominal ultrasonography in a routine health evaluation. There was no history of constitutional symptoms, overseas travel, intravenous drug ingestion, trauma or alcohol abuse. Physical examination showed no hepatomegaly, splenomegaly or lymphadenopathy and laboratory findings were unremarkable without slight elevation of sIL-2R (soluble interleukin 2 receptor) level. Abdominal US showed a hypoechoic splenic mass, and enhanced abdominal CT scan confirmed the existence of a low density, hypovascular, well-defined, round, smooth mass measuring 3.5×3.0 cm (Figure 1A and B). Abdominal MRI also revealed the splenic mass which showed low to isointensity on T1-weighted image (Figure 1C) and irregular intensity on T2-weighted image (Figure 1D). However, no lymph node swelling was detectable by imaging modalities. Under a diagnosis of a malignant lymphoma, laparotomic splenectomy was performed. On abdominal findings, the mass was not adherent to surrounding structures and other organs had not been invaded and finally, the spleen was removed without any accidents. Macroscopically, the mass was round, well-circumscribed, solid, medullary and measured $3.5 \times 3.0 \times 3.0$ cm, and the
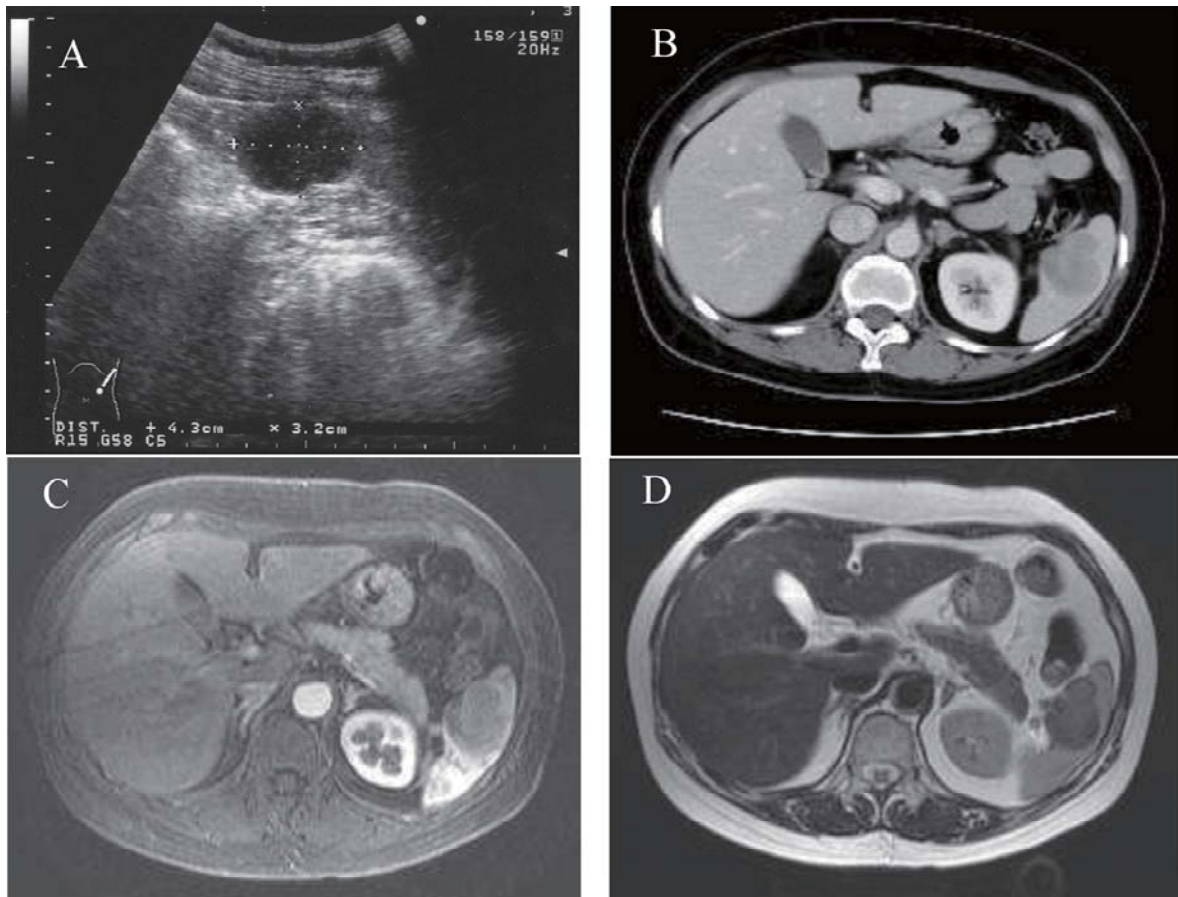
Figure 1. Preoperative findings by imaging modalities. A, US shows hypoechoic 4.3 × 3.2 cm mass in spleen; B, Enhanced CT scan showed intrasplenic homogeneous low-density 3.5-cm mass in the spleen; C and D, Both T1 and T2-weighted MRI shows heterogeneous low-signal intensity mass in spleen.
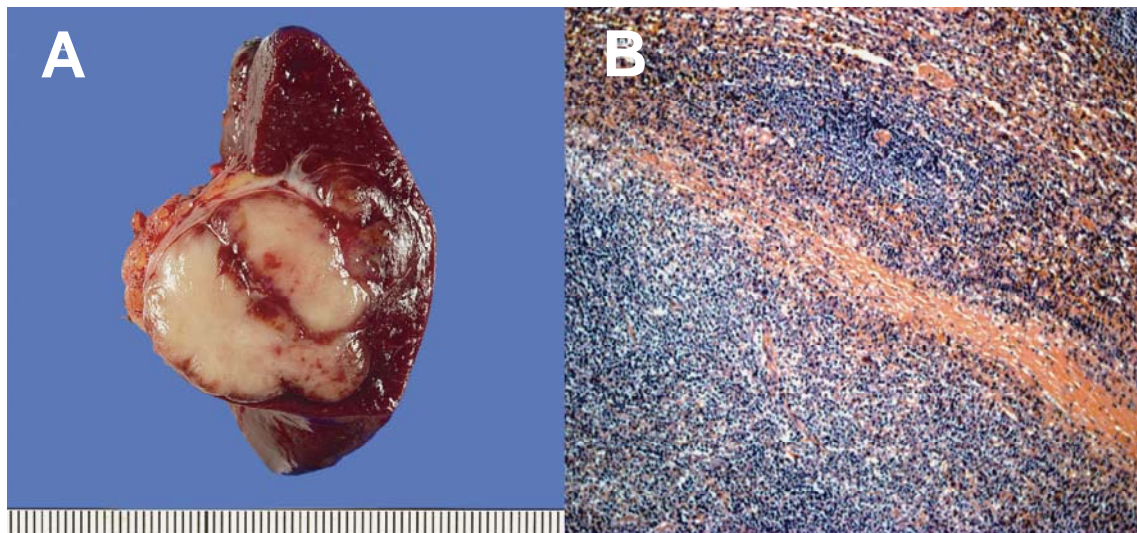
Figure 2. Pathological findings of the inflammatory splenic tumor. A, Cut surface of pseudotumor of spleen was well-circumscribed, and the center of mass showed milky white color; B, Photomicrography shows hyalized medullary lesion with filtration of lymphocytes and plasma cells (× 100).

center of mass had a milky white color (Figure 2A). Hisotological findings showed that the mass was infiltrated by many lymphocytes, plasma cells with a fibrous change. No abnormal infiltrated lymphocytes were detected and immunohistochemistrial stain on light chain showed mixed κ,λ chain positive cells. Also it was seen that follicular dendritic cells (FDC) in which Epstein-Barr virus (EBV) was detected by *in situ* hybridization with probes for EBER (small EBV RNA molecules) increased, leading us to diagnose an IPT of

the spleen (Figure 2B). The postoperative course was uneventful and the patient is currently asymptomatic, 3 years after surgery.

Discussion

IPT of the spleen are rare lesions that are usually discovered incidentally. The clinical symptoms are mostly diverse and some patients complain of pain in the left flank or left upper quadrant, with or without fever and splenomegaly, while others are asymptomatic. Laboratory data may show hypercalcemia (*7*), monoclonal peaks in the proteinogram (*8*) and polyclonal hypergammaglobulinemia (*9*) that disappear after splenectomy.

Recent advances in imaging with ultrasound, CT scan, and MRI are helpful in the identification of space occupying lesions of the spleen, and differential diagnosis must be made such as abscess, hemangioma, angiosarcoma, malignant lymphoma and hamartoma (*8*). Splenic abscesses are considered less likely because of lack of inflammatory response or fluid component. The lack of calcification and fatty elements argues against splenic hemangioma or angiosarcoma, whereas the lack of a cystic configuration argues against lymphangioma. As a result, primary splenic lymphoma or hamartoma remain in the differential diagnosis (*10*). Most frequently CT, US and MRI have been used to detect IPT of the spleen in previously reported cases, which shows noncharacteristic findings that are homogeneous low-density area in enhanced CT, heterogeneous low intensity mass in MRI (T2-weighted image). However, these findings were not specific to differentiate this type of lesion from other neoplasms (*9,12,13*). Some insist that histopathologic examination of a specimen obtained using sonographically guided Tru-cut needle biopsy can reveal IPT of the spleen. But needle biopsy has uncertainness of detection of the disease, risk of metastases if the mass is a malignant neoplasm and potential hemorrhagic complications of the procedure. Therefore, histological examination of resected specimens is the gold standard for diagnosing tumors of the spleen (*8*).

The microscopic findings are characteristic and IPT can be diagnosed by identifying the reactive nature of the cells. An IPT of the spleen shows some resemblance to granulation tissue, normal lymphocytes and plasma cells are constant features and should be distinguished from two other tumors: the IPT-like FDC tumor, which is consistently associated with EBV; and an inflammatory myofibroblastic tumor. These two lesions are neoplastic and therefore have a potentially worse prognosis than IPT.

Although the pathogenesis of this entity is unknown, infections, vascular causes and autoimmune disorders have been hypothesized (*9,12,14,15*). Infection is thought to be one of the causes because of the presence

of granulomas and giant cells. Some cases were reported to be due to EBV-positive inflammatory FDC tumors (*16*). Vascular causes are another hypothesis of pathogenesis since these lesions may be due to intraparencymatous hemorrhage secondary to traumatism or coagulopathy. Cotelingam and Jaffe (*13*) suggested the main initial event may have been a focal parenchymal necrosis with hemorrhage. Another hypothesis of immunological origin is presumed from high content of plasma cells in this lesion (*17*). Someren supports this idea because this entity is histologically similar to processes of recognized autoimmune origin (*18*). In our case, the specimen consists of high density of plasma cells, no underlying vascular changes and FDC positive by EBER-*in situ* hybridization, we assume the mass resulted from the process of inflammation from cytokine induced by EBV infection.

According to the previously published cases, the prognosis of IPT of the spleen has generally been considered favorable after splenectomy. After removal of the lesion, there have been no reports of metastatic disease, local invasion or recurrence. However, careful follow-up after removal is necessary in case of neoplasm, since some patients with IPT of the liver are reported to have died probably as a result of the disease (*19*) and there have been some reports of IPT of the spleen containing a monoclonal population of EBV-infected tumor cells. The presence of clonal EBV DNA suggests that some IPT of the spleen may be true neoplasms (*1,20*).

In summary, we report a case of an IPT of the speen found during a physical exam. The establishment of the diagnosis of a splenic tumor is often difficult and such lesions are occasionally malignant. Therefore, when a splenic tumor is found, it should be resected.

Acknowledgements

References

1. Oz Puyan F, Bilgi S, Unlu E, Yalcin O, Altaner S, Demir M, Cakir B. Inflammatory pseudotumor of the spleen with EBV positivity: report of a case. Eur J Haematol 2004;72:285-291.
2. Koh MB, Chae HS, Oh YK, Lee BO, Hur WH, Jung JH, You CR, Cho YS, Kim SS, Han SW, Lee CD, Choi KY, Chung IS, Ku YM. A case inflammatory pseudotumor of the spleen. Korean J Gastroenterol 2003;42:168-171.
3. Torzilli G, Inoue K, Midorikawa Y, Hui AM, Takayama T, Makuuchi M. Inflammatory pseudotumors of the liver: prevalence and clinical impact in surgical patients. Hepatogastroenterology 2001;48:1118-1123.
4. Yano H, Imasato M, Monden T, Okamoto S. Inflammatory pseudotumor of the spleen: Report of two cases. Surgery 2003;133:349-350.
5. Natsugoe S, Ohwaki T, Tsubouti H, Mitsuda K,

Maenohara S, Takao S, Aikou T, Shimazu H, Hasui K. Inflammatory pseudotumor of the spleen: report of a case. Surg Today 1993;23:246-250.

6. Noguchi H, Kondo H, Kondo M, Shiraiwa M, Monobe Y. Inflammatory pseudotumor of the spleen: A case report. Jpn J clin Oncol 2000;4:196-203.

7. Shepherd JJ, Fassett RG, Challis D. Hypercalcaemia associated with inflammatory pseudotumor of the spleen. Br J Surg 1992;79:50.

8. Galindo Gallego M, Ortega Serrano MP, Ortega Lopez M, Esteban Collazo F, Guinea Esquerdo L. Inflammatory pseudotumor of spleen. Report of two cases and literature review. Minerva Chir 1997;52:1379-1388.

9. Dalal BI, Greenberg H, Quinonez GE, Gough JC. Inflammatory pseudotuomr of the spleen. Morphological, radiological, immunophenotypic and ultrastructural features. Arch Pathol Lab Med 1991;115:1062-1064.

10. Suga K, Miura K, Kume N, Hara A, Matsunaga N, Tangoku A, Oka M. Tc-99m colloid and Ga-67 imaging of splenic inflammatory pseudotumor. Clin Nucl Med 1999;24:334-337.

11. Wiernik PH, Rader M, Becker NH, Morris SF. Inflammatory pseudotumor of spleen. Cancer 1990; 66:597-600.

12. Franquet T, Montes M, Aizcorbe M, Barberena J, Aazua YR, Cobo F. Inflammatory pseudotumor of the spleen: ultrasound and computed tomographic findings. Gastrointest Radiol 1989;14:181-183.

13. Cotelingam JD, Jaffe ES. Inflammatory pseudotumor of the spleen. Am J Surg Pathol 1984;8:375-380.

14. Perrone T, De Wolf-Peeters C, Frizzera G. Inflammatory pseudotumor of lymph nodes. A distinctive pattern of nodal reaction. Am J Surg Pathol 1988;12:351-361.

15. Sheahan K, Wolf BC, Neiman RS. Inflammatory pseudotumor of the spleen: a clinicopathologic study of three cases. Hum Pathol 1988;19:1024-1029.

16. Grouls V. Pseudolymphoma (inflammatory pseudotumor) of the liver. Zentralbl Allg Pathol 1987;133:565-568.

17. Chan JKC. Inflammatory pseudotumor: a family of lesions of diverse nature and etiologies. Am J Surg Pathol 1995;19:859-872.

18. Someren A. 'Inflammatory pseudotumor' of the liver with occlusive phlebitis. Report of a case in a child and review of the literature. Am J Clin Pathol 1978;69:176-181.

19. Horiuchi R, Uchida T, Kojima T, Shikata T. Inflammatory pseudotumor of the liver: clinicopathologic study and review of the literature. Cancer 1990;65:1583-1590.

20. Dominis M, Dzebro S, Kusic B, Antica M. Inflammatory pseudotumor of the spleen. Acta Cytol 1998;42: 1053-1056.

BioScience Trends

Guide for Authors

1. Scope of Articles

BioScience Trends aims to publish accessible material that will encourage cooperation and exchange among scientists and clinical researchers. Studies on public heath, the medical care system, and social science are also within the scope of BioScience Trends.

2. Submission Types

Original Articles should be reports on new, significant, innovative, and original findings. An Article should contain the following sections: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, References, Figure legends, and Tables. There are no specific length restrictions for the overall manuscript or individual sections. However, we expect authors to present and discuss their findings concisely.

Brief Reports should be short and clear reports on new original findings and not exceed 4000 words with no more than two display items. BioScience Trends encourages younger researchers and doctors to report their research findings. Case reports are included in this category. A Brief Report contains the same sections as an Original Article, but Results and Discussion sections must be combined.

Mini-Reviews should include educational overviews for general researchers and doctors and review articles for more specialized readers. Mini-Reviews should not exceed 8,000 words.

Policy Forum presents issues in science policy, including public health, the medical care system, and social science. Policy Forum essays should not exceed 2,000 words.

News articles should not exceed 500 words including one display item. These articles should function as an international news source with regard to topics in the life and social-sciences and medicine. Submissions are not restricted to journal staff anyone can submit news articles on subjects that would be of interest to BioScience Trends readers.

Letters discuss material published in BioScience Trends in the last 6 months or issues of general interest. Letters should not exceed 800 words.

3. Manuscript Preparation

Preparation of text. Manuscripts should be written in correct American English and submitted as a Microsoft Word (.doc) file in a single-column format. Manuscripts must be paginated and double-spaced throughout. Use Symbol font for all Greek characters. Do not import the figures into the text file but indicate their approximate locations directly on the manuscript. The manuscript file should be smaller than 5 MB in size.

Title page. The title page must include 1) the title of the paper, 2) name(s) and affiliation(s) of the author(s), 3) a statement indicating to whom correspondence and proofs should be sent along with a complete mailing address, telephone/fax numbers, and e-mail address, and 4) up to five key words or phrases.

Abstract. A one-paragraph abstract consisting of no more than 250 words (200 words in Policy Forum essays) must be included. It should state the purpose of the study, basic procedures used, main findings, and conclusions.

Abbreviations. All nonstandard abbreviations must be listed in alphabetical order, giving each abbreviation followed by its spelled-out version. Spell out the term upon first mention and follow it with the abbreviated form in parentheses. Thereafter, use the abbreviated form.

Introduction. The introduction should be a concise statement of the basis for the study and its scientific context.

Materials and Methods. Subsections under this heading should include sufficient instruction to replicate experiments, but well-established protocols may be simply referenced. BioScience Trends endorses the principles of the Declaration of Helsinki and expects that all research involving humans will have been conducted in accordance with these principles. All laboratory animal studies must be approved by the authors' Institutional Review Board(s).

Results. The results section should provide details of all of the experiments that are required to support the conclusions of the paper. If necessary, subheadings may be used for an orderly presentation. All figures, tables, and photographs must be referred in the text.

Discussion. The discussion should include conclusions derived from the study and supported by the data. Consideration should be given to the impact that these conclusions have on the body of knowledge in which context the experiments were conducted. In Brief Reports, Results and Discussion sections must be combined.

Acknowledgments. All funding sources should be credited in the Acknowledgments section. In addition, people who contributed to the work but who do not fit the criteria for authors should be listed along with their contributions.

References. References should be numbered in the order in which they appear in the text. Cite references in text using a number in parentheses. Citing of unpublished results and personal communications in the reference list is not recommended but these sources may be mentioned in the text. For all references, list all authors, but if there are more than fifteen authors, list the first three authors and add "*et al.*" Abbreviate journal names as they appear in PubMed. Web references can be included in the reference list.

Example 1:
Ishizawa T, Hasegawa K, Sano K, Imamura H, Kokudo N, Makuuchi M. Selective versus total biliary drainage for obstructive jaundice caused by a hepatobiliary malignancy. Am J Surg 2007; 193:149-154.

Example 2:
Mizuochi T. Microscale sequencing

of *N*-linked oligosacchaides of glycoproteins using hydrazinolysis, Bio-Gel P-4, and sequential exoglycosidase digestion. In: Methods in Molecular Biology: Vol. 14 Glycoprotein analysis in biomedicine (Hounsell T, ed.). Humana Press, Totowa, NJ, USA, 1993; pp. 55-68.

Example 3:
BioScience Trends. Hot topics & news: China-Japan Medical Workshop on Drug Discoveries and Therapeutics 2007. http://www.biosciencetrends.com/hotnews.php (accessed July 1, 2007).

Figure legends. Include a short title and a short explanation. Methods described in detail in the Materials and Methods section should not be repeated in the legend. Symbols used in the figure must be explained. The number of data points represented in a graph must be indicated.

Tables. All tables should have a concise title and be typed double-spaced on pages separate from the text. Do not use vertical rules. Tables should be numbered with Roman numerals consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with lowercase superscript letters.

Language editing. Manuscripts submitted by authors whose primary language is not English should have their work proofread by a native English speaker before submission. The Editing Support Organization can provide English proofreading, Japanese-English translation, and Chinese-English translation services to authors who want to publish in BioScience Trends and need assistance before submitting an article. Authors can contact this organization directly at http://www.iacmhr.com/iac-eso .
    IAC-ESO was established in order to facilitate manuscript preparation by researchers whose native language is not English and to help edit work intended for international academic journals. Quality revision, translation, and editing services are offered by our staff, who are native speakers of particular languages and who are familiar with academic writing and journal editing in English.

4. Figure Preparation

All figures should be clear and cited in numerical order in the text. Figures must fit a one- or two-column format on the journal page: 8.3 cm (3.3 in.) wide for a single column; 17.3 cm (6.8 in.) wide for a double column; maximum height: 24.0 cm (9.5 in.). Only use the following fonts in the figure: Arial and Helvetica. Provide all figures as separate files. Acceptable file formats are JPEG and TIFF. Please note that files saved in JPEG or TIFF format in PowerPoint lack sufficient resolution for publication. Each Figure file should be smaller than 10 MB in size. Do not compress files. A fee is charged for a color illustration or photograph.

5. Online Submission

Manuscripts should be submitted to BioScience Trends online at http://www.biosciencetrends.com. The manuscript file should be smaller than 10 MB in size. If for any reason you are unable to submit a file online, please contact the Editorial Office by e-mail: office@ biosciencetrends.com .

Editorial and Head Office
Wei TANG, MD PhD
Secretary-in-General
TSUIN-IKIZAKA 410
2-17-5 Hongo, Bunkyo-ku
Tokyo 113-0033
Japan
Tel: 03-5840-8764
Fax: 03-5840-8765
E-mail: office@biosciencetrends.com

Cover letter. A cover letter from the corresponding author including the following information must accompany the submission: name, address, phone and fax numbers, and e-mail address of the corresponding author. This should include a statement affirming that all authors concur with the submission and that the material submitted for publication has not been previously published and is not under consideration for publication elsewhere and a statement regarding conflicting financial interests.
    Authors may recommend up to three qualified reviewers other than members of Editorial board. Authors may also request that

certain (but not more than three) reviewers not be chosen.
    The cover letter should be submitted as a Microsoft Word (.doc) file (smaller than 1 MB) at the same time the work is submitted online.

6. Accepted Manuscripts

Proofs. Rough galley proofs in PDF format are supplied to the corresponding author via e-mail. Corrections must be returned within 3 working days of the proofs. Subsequent corrections will not be possible, so please ensure all desired corrections are indicated. Note that we may proceed with publication of the article if no response is received.

Offprints. Fifty (50) offprints of each article are sent to the corresponding author. Additional offprints can be ordered before the article is published. Offprint requests after publication will be subject to an extra charge.

Page and color charges. A page charge of $140 will be assessed for each printed page of accepted manuscripts and $30 will be charged as a flat fee for each publication. The charge for printing of color figures is $340 for each page.

Transfer of copyrights. Upon acceptance of an article, authors will be asked to agree to a transfer of copyright. This transfer will ensure the widest possible dissemination of information. A letter will be sent to the corresponding author confirming receipt of the manuscript. A form facilitating transfer of copyright will be provided. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article.

Cover submissions. Authors whose manuscripts are accepted for publication in BioScience Trends may submit cover images. Color submission is welcome. A brief cover legend should be submitted with the image.

Revised July 2007

BioScience Trends

JOURNAL PUBLISHING AGREEMENT

Ms No:

Article entitled:

Corresponding author:

To be published in BioScience Trends

Assignment of publishing rights:

I hereby assign to International Research and Cooperation Association for Bio & Socio-Sciences Advancement (IRCA-BSSA) publishing BioScience Trends the copyright in the manuscript identified above and any supplemental tables and illustrations (the articles) in all forms and media, throughout the world, in all languages, for the full term of copyright, effective when and if the article is accepted for publication. This transfer includes the rights to provide the article in electronic and online forms and systems.

I understand that I retain or am hereby granted (without the need to obtain further permission) rights to use certain versions of the article for certain scholarly purpose and that no rights in patent, trademarks or other intellectual property rights are transferred to the journal. Rights to use the articles for personal use, internal institutional use and scholarly posting are retained.

Author warranties:

I affirm the author warranties noted below.
1) The article I have submitted to the journal is original and has not been published elsewhere.
2) The article is not currently being considered for publication by any other journal. If accepted, it will not be submitted elsewhere.
3) The article contains no libelous or other unlawful statements and does not contain any materials that invade individual privacy or proprietary rights or any statutory copyright.
4) I have obtained written permission from copyright owners for any excerpts from copyrighted works that are included and have credited the sources in my article.
5) I confirm that all commercial affiliations, stock or equity interests, or patent-licensing arrangements that could be considered to pose a financial conflict of interest regarding the article have been disclosed.
6) If the article was prepared jointly with other authors, I have informed the co-authors(s) of the terms of this publishing agreement and that I am signing on their behalf as their agents.

Your Status:

☐ I am the sole author of the manuscript.

☐ I am one author signing on behalf of all co-authors of the manuscript.

Please tick one of the above boxes (as appropriate) and then sign and date the document in black ink.

Signature: **Date:**

Name printed:

Please return the completed and signed original of this form by express mail or fax, or by e-mailing a scanned copy of the signed original to:

BioScience Trends office
TSUIN-IKIZAKA 410, 2-17-5 Hongo,
Bunkyo-ku, Tokyo 113-0033, Japan
E-mail: proof-editing@biosciencetrends.com
Fax: +81-3-5840-8765