Annual Report

2015

NCGM-BMH
Medical Collaboration Center

March 2016
Tokyo, Japan-Hanoi, Viet Nam

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Preface

At first, congratulations for 105-year Anniversary of Bach Mai Hospital!

This is our pleasure that we can continue our collaboration between Bach Mai Hospital (BMH), Viet Nam and National Center for Global Health and Medicine (NCGM), Japan.

We are conducting collaborative research activities and other human resource development activities. In addition, it is a great news that “Sister Renal Center Program” funded by International Society of Nephrology is upgraded because of the great efforts of nephrology sections of both side. Furthermore, in the Year 2015, “the Program for International Promotion of Japan’s Healthcare Technologies and Services” has been launched. This is the new cooperation scheme funded by the Ministry of Health, Labor and Welfare (MHLW) of Japan, through “the Program for International Promotion of Japan’s Healthcare Technologies and Services”, and NCGM is designated as the organizer of this new scheme by the MHLW. Based on this new scheme, we can expect that the clinical collaboration can be accelerated more in the next year as well.

Finally, we would like to express our congratulation to BMH’s opening of new hospital building, as well. We wish our further collaboration between us in the new year, 2016.

March, 2016

Hidechika Akashi, MD, PhD, MPH, DTMH
Director,
Medical Collaboration Center (MCC)
National Center for Global Health and Medicine (NCGM), Japan

Abbreviations

BMH	Bach Mai Hospital
NCGM	National Center for Global Health and Medicine
IMCJ	International Medical Center of Japan
MCC	NCGM - BMH Medical Collaboration Center
MOH	Ministry of Health, Viet Nam
MEXT	Ministry of Education, Culture, Sport, Science and Technology, Japan
J-GRID	Japan Initiative for Global Research Network on Infectious Diseases
MHLW	Ministry of Health, Labor and Welfare, Japan
JICA	Japan International Cooperation Agency
MOU	Memorandum of Understanding
HCMC	Ho Chi Minh City
NIHE	National Institute of Hygiene and Epidemiology
NHP	National Hospital of Pediatrics
NLH	National Lung Hospital
HLH	Hanoi Lung Hospital
NIHBT	National Institute of Hematology and Blood Transfusion
RIT-JATA	Research Institute of Tuberculosis-Japan Anti-Tuberculosis Association
WHO	World Health Organization
JFPIMRC	Japan Foundation for the Promotion of International Medical Research Cooperation
SARS	Severe Acute Respiratory Syndrome
DCC	Disease Control and Prevention Center of NCGM

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I. General information on NCGM-BMH Medical Collaboration Center (MCC)

1. Background

Since the beginning of 1990's, National Center for Global Health and Medicine (NCGM) (former IMCJ) has been carrying out important roles in collaboration with health sector in Viet Nam for the purpose of the improvement of medical situation in the country. Particularly, collaboration with Bach Mai Hospital (BMH) has been implemented most actively and effectively. In the grant-aid and the technical cooperation projects in BMH, which was supported by Japan International Cooperation Agency (JICA), NCGM contributed to the successful implementation by dispatching experts and providing technical guidance.

Through the history of the past collaboration, NCGM has established close and reliable relationship with BMH and other leading medical institutions in Viet Nam. Using these bases, a new collaboration, which is conducted distinctly from ODA projects and focusing on research and human resource development, was designed.

In order to implement the new collaborative activities, the NCGM-BMH Medical Collaboration Center (MCC) was planed.

2. Establishment of MCC

In view of the successful outcome of BMH project (phase 1) and the efficient collaboration during the SARS outbreak in 2003, a plan to establish a medical collaboration center between NCGM and BMH, which functions separately from JICA projects, grew up in NCGM. The idea was put into practice when the research project on emerging and reemerging infectious diseases was proposed by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT).

In recent years, emerging and reemerging infectious diseases have been threatening the world. In view of the rising fear of these diseases, the MEXT launched a new project in world-wide scale to cope with emerging and reemerging infectious diseases efficiently by setting up medical collaboration centers and conducting close collaboration there. The proposal of the MEXT project facilitated the realization of MCC in Viet Nam. After several preliminary studies, NCGM and BMH decided to establish MCC within BMH based on the friendly and reliable relationship which had been developed since the beginning of 1990's between the 2 medical institutions.

The Memorandum of Understanding (MOU) regarding the initiation of the project was signed by the Director of BMH and the President of NCGM in August 2005 followed by the official approval of the Ministry of Health, Viet Nam. In April 2010, NCGM changed its name (from International Medical Center of Japan; IMCJ to National Center for Global Health and Medicine; NCGM) due to its organizational reform (Independent Administrative Legal Entity). In view of this situation, both sides agreed to revise the MOU along with continuation of the current cooperative activities. After discussions between NCGM and BMH the revised MOU was drafted. In the new MOU, activities in MCC are clarified as research, training, medical case conference, technical cooperation, international conference/ meeting/seminars, personal exchange programs, and others, although in the current

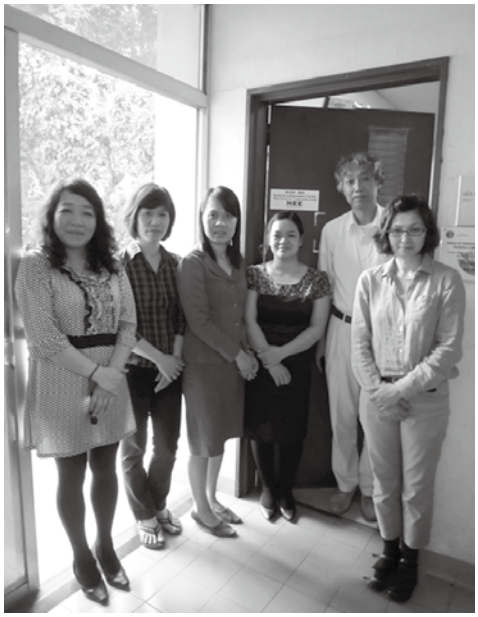
Signing ceremony of the Memorandum of Understanding between NCGM and BMH in 2010

version description of activity is concentrated on researches. The new MOU, after getting approval of MOH, was signed by the representatives of BMH (Dr. Nguyen Quoc Anh) and NCGM (Dr. Takaaki Kirino) in June 2, 2010.

MCC office was established in the new building of BMH, which was constructed by Japan's grant-aid in 2000, as the managing center of various collaborative activities including the MEXT project and others. Based on the MCC, various activities were started in collaboration with BMH along with related medical institutions.

3. Objective of MCC

The objective of setting up MCC in Viet Nam is to implement various collaboration on medical science and medical care, such as researches, human resource development & technical exchange, information sharing, clinical case conference, etc. smoothly and effectively. The activities in MCC are conducted in close collaboration between BMH and NCGM, and related medical institutions and such collaborative activities are expected to benefit both Viet Nam and Japan. The contents of activities can include some advanced and sophisticated techniques which had been difficult to conduct within the framework of JICA projects.



4. Related medical institutions

MCC is mainly collaborating with BMH, however based on the agreement described in MOU; some related medical institutions have been setting up under the approval of BMH.

Currently, five institutions in Hanoi and three institutions in Ho Chi Minh City are functioning as the main related medical institutions. In the future, more medical institutions might be added if necessary and efficient network building among them of are expected.

Table 1: Main medical institutions under collaboration (As of December 2015)

No.	Medical institution	Location	Collaborative study
1	National Institute of Tropical and Infectious Diseases	Hanoi	HIV/AIDS
2	National Hospital of Pediatrics	Hanoi	Clinical conference
3	National Lung Hospital (the former National Hospital of Tuberculosis and Lung Diseases)	Hanoi	Tuberculosis
4	Hanoi Lung Hospital (the former Hanoi Hospital of Tuberculosis and Lung Diseases)	Hanoi	Tuberculosis
5	Ho Chi Minh Medical and Pharmaceutical University	HCMC	Tropical Medicine
6	Ho Chi Minh City Hospital of Tropical Medicine	HCMC	Tropical Medicine Medical education
7	National Institute of Nutrition	Hanoi	Diabetes and life style related disease
8	Cho Ray hospital	HCMC	Training program

5. MEXT project

The Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) is implementing the MEXT "Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases" in Asian and African countries. The objective of these activities is to contribute to the emerging infectious diseases and other disease control from the world-wide viewpoint. As of December 2008, this project has been implemented in 11 research centers in 8 countries (Viet Nam, China, India, Thailand, Indonesia, Zambia, Ghana, Philippines). MCC is functioning as one of the important research centers for this project in Viet Nam. The period of this project is 5 years from April 2005 to March 2010. The next step started from April 2010 to March 2015, and now the third step has been started from April 2015.

Currently, the project supported by MEXT accounts for the major part of the activities of MCC. Activities of the MEXT project in Viet Nam including researches (both basic and clinical researches), human resource development, etc. Equipment which is needed to conduct these activities effectively has also been provided to BMH and relevant medical institutions.

MEXT project in Viet Nam consists of the following three research groups (Dr. Oka is the leader of these researches). These groups are implementing activities on emerging and reemerging infectious disease control based on the concept of MEXT project. The following three researches are three leading research subjects in MCC and under these research themes, sub-researches have been carried out.

1) Dr. Oka's group: HIV/AIDS
2) Dr. Ohmagari's group: Bacterial infections
3) Dr. Keicho's group: Tuberculosis

6. Other projects

In addition to MEXT and the new Program, the Ministry of Health, Labor and Welfare of Japan (MHLW) is also supporting research projects on various fields. As an oversea base of NCGM, MCC is functioning as a body to support NCGM research teams or individuals who want to implement a collaborative project in Viet Nam.

Within this scope, life-style related diseases such as diabetes and pediatric health issues are projects which have been implemented in collaboration with BMH and other medical institutions of Viet Nam so far. A community-based survey on diabetes and obesity, followed by an intervention program has been implementing in Hanoi area and a lot of meaningful data have been obtained with the support from MCC.

In 2013, the Sister Renal Center Program was applied in cooperation between Nephro-Urology Department, BMH and Nephrology Department, NCGM to receive the support from the International Society of Nephrology. Under this program, in 2015, two Japanese groups including NCGM nephrologists came to BMH for training courses on kidney diseases and quality management of water for hemodialysis, with the trainees from BMH and other hospitals. Three staff of the Nephro-Urology Department, BMH were invited for training in NCGM. Together with the Bureau of International Medical Cooperation of NCGM, MCC participated in supporting the implementation of this program.

7. The program for International Promotion of Japan's Healthcare Technologies and Services

The new program, "The Program for International Promotion of Japan's Healthcare Technologies and Services", has been started from 2015, organized by the Bureau of International Health Cooperation (former "Bureau of International Medical Cooperation") and relevant departments of NCGM and funded by MHLW,

Japan. This program is in relation to the Memorandum of Cooperation in the field of healhcare between MHLW of Japan and the Ministry of Health of Viet Nam signed on 18 March 2014 by both Ministers. This program aims at carrying out the major objectives of promotion, including sharing the experiences in Japan's public health insurance system and the transfer of cutting-edge medical technologies. Through this program, public health standards in the counterpart countries will be improved.

Under this program, nine staff of BMH were invited to NCGM for training in different fields, such as clinical laboratory, quality management, nursing management etc. Healthcare staff working in quality management and patient safety in other related hospitals were also invited to NCGM for training. Specialists from NCGM also came to BMH for investigating the needs and discussing the content of training and collaborative activities.

8. Current MCC

In 2015, MCC has received many groups of researchers and specialists coming from NCGM and related institutions in Japan. In addition to logistic support for these groups, such as reservation of accommodation, arrangement of transportation vehicles, on-site coordination including making appointments with Viet Namese counterparts, MCC staff also participated in discussions on related activities held between the two sides. Together with Viet Namese counterparts, MCC staff also took part in conducting and monitoring on-site implementation of researches, as well as collecting and reporting data.

MCC staff also supported administrative procedures for Viet Namese counterparts, who were invited to Japan for training. In 2015, nearly 20 counterpart members have completed necessary procedures and successfully received training in Japan. Through this activity, MCC also acts as a bridge to link domestic health personnel and institutions, which is necessary for sustainable improvement.

In September 2015, MCC participated in arrangement of the training visits for NCGM trainees. Groups including doctors, nurses, technicians, and pharmacists came to BMH and other hospitals and healthcare centers in Hoa Binh province for the training on global health and medicine. In December 2015, MCC participated in arrangement of another group of visitors under the global nursing study tour conducted by NCGM. Some activities including nosocomial infection control and nursing education were included in this study tour.

II. Activities

1. Research

List of collaborative researches in MCC, Viet Nam

Table 2 Collaborative researches in MCC, Viet Nam

No.	Main Researcher in NCGM	Affiliation in Viet Nam	Subject	Source of fund
1	Shinichi Oka	National Hospital of Tropical Diseases (NHTD), Bach Mai Hospital(BMH)	The cohort study of HIV-1-infected individuals in Northern Viet Nam	AMED
2	Norio Ohmagari Nguyen Quoc Anh	Bach Mai Hospital(BMH)	Research on Epidemiology, Diagnosis and Treatment for Healthcare Associated Infection and Antimicrobial Resistant Bacteria in Viet Nam	J-GRID
3	Naoto Keicho Luu Thi Lien Pham Huu Thuong	Hanoi Lung Hospital (HLH) National Lung Hospital (NLH)	Research on tuberculosis in Viet Nam Research on spreading Beijing-genotype strains of *Mycobacterium tuberculosis*, their drug-resistance profiles and possible effects on treatment outcome	J-GRID MEXT
4	Hiroyuki Shichino	National Hue Central Hospital (Hue)	Support for Strengthening Medical Treatment Ability of the Childhood Cancer in Viet Nam	International Promotion of Japan's Healthcare Technologies and Services, NCGM Program, MHLW(A27-5)
5	Junko Yamanaka	National Hue Central Hospital (Hue)	Improving the quality of medical treatment for childhood cancer in developing countries	International Health Research and Development Fund (25-13)
6	Kajio H Anh NQ Lien DTK	Bach Mai Hospital(BMH)	Impact of a life style intervention in incident and prevalence of overweight and obesity among secondary school children in Hanoi	NCGM MHLW
7	Hiroshi Ohara	National Institute of Malariology, Parasitology and Entomology (NIMPE), Bach Mai Hospital(BMH)	Assessment of the interface between malaria control program and health system strengthening	NCGM (27-4, 24-5)
8	Naoto Keicho Ngo Quy Chau Le Cong Dinh	Bach Mai Hospital(BMH)	A study on sinopulmonary disease (chronic rhinosinusitis with chronic lower airway infection) in Viet Nam	NCGM

No.	Main Researcher in NCGM	Affiliation in Viet Nam	Subject	Source of fund
9	Naoto Keicho	NCGM-BMH Medical Collaboration Center	Research on establishing basis of international collaborative studies in Viet Nam	NCGM
10	Toshie Manabe Ngo Quy Chau Koichiro Kudo	Bach Mai Hospital(BMH)	Chronological, geographical, and seasonal trends of human cases of avian influenza A (H5N1) in Viet Nam, 2003–2014: a spatial analysis	J-GRID
11	Nguyen Gia Binh Vu Thi Tuong Van Noriko Nakajima Tsutomu Kageyama Jin Takasaki	Bach Mai Hospital(BMH)	International collaborative study for diagnosis of avian influenza virus infection and other respiratory viral infections	AMED

Research No.1

1.	**Title(in English)**	The cohort study of HIV-1-infected individuals in Northern Viet Nam
2.	**Title(in Japanese)**	ハノイにおける HIV 感染者のコホート研究
3.	**Main researcher**	Shinichi Oka (AIDS Clinical Center, National Center for Global Health and Medicine)
4.	**Co-Researcher(s)**	Junko Tanuma, Daisuke Mizushima, Ei Kinai, Hiroyuki Gatanaga, Tsunefusa Hayashida, Shoko Matsumoto, Masafumi Takiguchi, Nguyen Thi Huyen,Nguyen Hoai Dung, Nguyen Tien Lam, Nguyen Van Kinh, Pham Thi Thanh Thuy, Vu Thi Tuong Van, Doan Thu Tra, Nguyen Quang Tuan, Do Duy Cuong
5.	**Resource of fund**	Japan Agency for Medical Research and Development (AMED)
6.	**Affiliation(s) in Viet Nam**	National Hospital of Tropical Diseases (NHTD) Bach Mai Hospital (BMH)
7.	**Period of the research**	October 2007- March 2020
8.	**Publications**	1. Kinai E. et al. ***JAIDS*** in press 2. Matsumoto S. et al. ***PLOS One*** 10(9) e0139594, 2015. 3. Sawada I, et al. ***PLOS One*** 10 (4): e0125299, 2015. 4. Kuse N, et al. ***J Virol*** 89 (14): 7363-7372, 2015. 5. Tran GV, et al. ***AIDS*** 2015 Nov 20. [Epub ahead of print]

9. Summary:

Prospective research cohorts of HIV-infected persons have made a major contribution to an understanding of the transmission, natural history and pathogenesis of HIV infection, in addition to generating important information on the response to and long-term outcomes with antiretroviral therapy (ART). Under the project of Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), we have established a hospital-based cohort of HIV-infected individuals in the National Hospital of Tropical Diseases (NHTD) since October 2007 in Hanoi, and the Infectious Disease Department in Bach Mai Hospital has joined the cohort since December 2011, which enables us to follow up patients prospectively with standardized methods of data collection at regular defined time points, for the purpose of clinical researches on HIV/AIDS focused on Asian population.

From October 2007 through December 2015, the demographic, clinical and laboratory data had been collected on HIV-positive patients seen at NHTD and BMH in Hanoi, Viet Nam. The data collection occurs every 6 months and the data has been stored and managed in a relational database, which was originally created for the East Asia Clinical HIV Cohort (HIV cohort in Japan, Korea and Singapore) and modified for the Hanoi HIV Cohort, enabling us combined analysis of the two cohorts. By the end of December 2015, we recruited 1359 HIV-positive patients on ART and 461 ART-naïve patients in NHTD and 378 HIV-positive patients on ART in BMH to the Hanoi cohort. 63.1% of the cohort was male, and the median age was 32 years at first follow-up. The risk factors for HIV infection were sex between men and women (72.8%) and injection drug use (30.8%).

The cohort would provide important information on the status of HIV-infected individuals in Viet Nam and a variety of opportunities to study the unique characteristics on the pathogenesis or the treatment outcome of HIV infection in Asian population.

Research No.2

1	Title (in English)	Research on Epidemiology, Diagnosis and Treatment for Healthcare Associated Infection and Antimicrobial Resistant Bacteria in Viet Nam
2	Title (in Japanese)	ベトナム拠点における医療関連感染症及び後期若耐性菌感染症に関する検討
3	Main researcher	Norio Ohmagari MD., MSc (Director, Disease Control and Prevention Center, National Center for Global Health and Medicine) Nguyen Quoc Anh MD., PhD. (Director, Bach Mai Hospital)
4	Co-Researcher(s)	Teruo Kirikae, MD, PhD (NCGM), Tohru Miyoshi-Akiyama, PhD (NCGM), Tatsuya Tada, PhD (NCGM), Nozomi Takeshita, MD., PhD (NCGM), Kayoko Hayakawa, MD., PhD (NCGM), Maki Nagamatsu (NCGM), Mitsuhiro Tsuchiya, MSc (NCGM), Pham Thi Phuong Thuy BA. MPH (NCGM-BMH Medical Coloration Center), Nguyen Gia Binh, MD., PhD, Doan Mai Phuong MD., PhD (Head of Microbiology Dept., Bach Mai Hospital), Do Van Thanh (Infectious Dept. and International Dept. Bach Mai Hospital)
5	Resource of fund	Japan Initiative for Global Research Network on Infectious Diseases (Funded from Ministry of Education, Science and Technology, Culture and Sport of Japan)
6	Affiliation(s) in Viet Nam	Bach Mai Hospital
7	Period of the research	April 1, 2012 to March, 2016
8	Publications	
9	Summary: 1. BMC Infect Dis. 2015 Oct 15;15:433. doi: 10.1186/s12879-015-1171-x. Dissemination of clonal complex 2 *Acinetobacter baumannii* strains co-producing carbapenemases and 16S rRNA methylase ArmA in Viet Nam. Tada T(1), Miyoshi-Akiyama T(2), Shimada K(3), Nga TT(4), Thu le TA(5), Son NT(6), Ohmagari N(7), Kirikae T(8). **Author information:** (1) Department of Infectious Diseases, Research Institute, National Center for Global Health and Medicine, Shinjuku, Tokyo, Japan. ttada@ri.ncgm.go.jp. (2) Pathogenic Microbe Laboratory, Research Institute, National Center for Global Health and Medicine, Shinjuku, Tokyo, Japan. takiyam@ri.ncgm.go.jp. (3) Department of Infectious Diseases, Research Institute, National Center for Global Health and Medicine, Shinjuku, Tokyo, Japan. kshima@ri.ncgm.go.jp. (4) Cho Ray Hospital, Ho Chi Minh, Viet Nam. ngatrancrh@gmail.com. (5) Cho Ray Hospital, Ho Chi Minh, Viet Nam. letathu@yahoo.com. (6) Cho Ray Hospital, Ho Chi Minh, Viet Nam. truongson_cr@yahoo.com.vn. (7) Disease Control and Prevention Center, Division of Infectious Diseases, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku, Tokyo, 162-8655, Japan. nohmagari@hosp.ncgm.go.jp. (8) Department of Infectious Diseases, Research Institute, National Center for Global Health and Medicine, Shinjuku, Tokyo, Japan. tkirikae@ri.ncgm.go.jp.	

BACKGROUND: *Acinetobacter baumannii* strains co-producing carbapenemase and 16S rRNA methylase are highly resistant to carbapenems and aminoglycosides.

METHODS: Ninety-three isolates of multidrug-resistant *A. baumannii* were obtained from an intensive care unit in a hospital in Viet Nam. Antimicrobial susceptibility tests and whole genome sequencing were performed. Multilocus sequence typing and the presence of drug resistant genes were determined and a maximum-likelihood phylogenetic tree was constructed by SNP alignment of whole genome sequencing data.

RESULTS: The majority of isolates belonged to clonal complex 2 (ST2, ST570 and ST571), and carried carbapenemase encoding genes bla OXA-23 and bla OXA-66. Two isolates encoded carbapenemase genes bla NDM-1 and bla OXA-58 and the 16S rRNA methylase encoding gene armA and did not belong to clonal complex 2 (ST16).

CONCLUSION: *A. baumannii* isolates producing 16S rRNA methylase ArmA and belonging to clonal complex 2 are widespread, and isolates co-producing NDM-1 and ArmA are emerging, in medical settings in Viet Nam.

2. Antimicrob Agents Chemother. 2015 Nov;59(11):7090-3. doi: 10.1128/AAC.01611-15.

IMP-51, a novel IMP-type metallo-β-lactamase with increased doripenem- and meropenem-hydrolyzing activities, in a carbapenem-resistant *Pseudomonas aeruginosa* clinical isolate.

Tada T(1), Nhung PH(2), Miyoshi-Akiyama T(3), Shimada K(1), Phuong DM(4), Anh NQ(4), Ohmagari N(5), Kirikae T(6).

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A meropenem-resistant *Pseudomonas aeruginosa* isolate was obtained from a patient in a medical setting in Hanoi, Viet Nam. The isolate was found to have a novel IMP-type metallo-β-lactamase, IMP-51, which differed from IMP-7 by an amino acid substitution (Ser262Gly). Escherichia coli expressing blaIMP-51 showed greater resistance to cefoxitin, meropenem, and moxalactam than E. coli expressing blaIMP-7. The amino acid residue at position 262 was located near the active site, proximal to the H263 Zn(II) ligand.

3. J Infect Chemother. 2015 Aug;21(8):617-9. doi: 10.1016/j.jiac.2015.04.002. Epub 2015 Apr 18.

Evaluation of the Etest method for detecting colistin susceptibility of multidrug-resistant Gram-negative isolates in Viet Nam.

Nhung PH(1), Miyoshi-Akiyama T(2), Phuong DM(3), Shimada K(2), Anh NQ(3), Binh NG(3), Thanh do V(3), Ohmagari N(4), Kirikae T(5).

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The minimum inhibitory concentrations (MICs) of colistin for 241 multidrug-resistant (MDR) Gram-negative pathogens were determined by the Etest and by the broth microdilution method (BMD). The two methods showed essential agreements of76% (77/102) for *Acinetobacter baumannii*, 90% (36/40) for *Pseudomonas aeruginosa* and 84% (83/99) for Enterobacteriaceae isolates, with categorical agreements of 100%, 98%, and 100%, respectively. Of the 241 isolates, none showed a very major error and one (0.4%) showed a major error. MICs ranged from 0.125 to 0.5 μg/ml for all *A. baumannii* and most Enterobacteriaceae isolates, and from 1 to 2 μg/ml for most *P. aeruginosa* isolates. Of the 40 *P. aeruginosa* isolates, 27 (68%) showed higher colistin MICs by the Etest than by the BMD. In contrast, 77% (78/102) of the *A. baumannii* and 57% (56/99) of the Enterobacteriaceae isolates showed lower colistin MICs by the Etest than by the BMD. The Etest is a reliable and easy-to-use method to measure colistin MICs of MDR Gram-negative pathogens in clinical laboratories and can be used following validation by microdilution methods.

4. Int J Infect Dis. 2015 Jun;35:18-23. doi: 10.1016/j.ijid.2015.03.020. Epub 2015 Mar 30.

The efficacy and nephrotoxicity associated with colistin use in an intensive care unit in Viet Nam: Use of colistin in a population of lower body weight.

Binh NG(1), Hayakawa K(2), Co DX(1), Tuan ND(1), Anh NH(3), Thuy NT(4), Phuong DM(5), Huong NT(3), Thuy PT(6), Chau NQ(7), Nhung PH(5), Gam do TH(4), Hai DT(4), Huong TT(4), Van Anh L(4), Takeshita N(8), Ohmagari N(8).

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(6) National Center for Global Health and Medicine - Bach Mai hospital Medical Collaboration Center.
(7) Respiratory Department, Bach Mai Hospital, Hanoi, Viet Nam.
(8) Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan.

BACKGROUND: There has been a growing need for colistin as a key drug for the treatment of MDR-GNB infection. Information on colistin use in Asian population is limited.

	METHODS: A retrospective observational study was conducted to assess the efficacy and nephrotoxicity in critically ill adult patients who received intravenous colistin for MDR-GNB infection in the intensive care unit (ICU) at Bach Mai Hospital in Hanoi, Viet Nam. Colistin was administered according to the dosing guideline that was based on pharmacokinetic, pharmacodynamic and toxicodynamic principles, adjusted by body weight and creatinine clearance. **RESULTS:** Twenty-eight eligible patients were included. The mean patient age was 60±20.4 years. The mean body weight was 53±8.6kg. The mean daily dose of colistin was 4.1±1.6 MIU, and the mean cumulative dose of colistin was 48.2±22.8 MIU. Colistin therapies were classified as clinically effective in 19 (67.9%) cases. Six (21.4%) patients developed nephrotoxicity during the study period accordingto RIFLE criteria. **CONCLUSION:** A personalized dosing protocol of colistin was effective, with low nephrotoxicity, among critically ill Viet Namese patients with low body weight. Further studies are warranted for assessing the efficacy and toxicity in a larger cohort.

Educational activities:
Training Course on Case Management of Tropical Infectious Diseases was held at Ho Chi Minh City, Viet Nam (November-December 2015). Three infectious diseases residents and one fellow from NCGM had participated in this training course.

Research No.3

1	Title (in English)	Research on tuberculosis in Viet Nam Research on spreading Beijing-genotype strains of *Mycobacterium tuberculosis*, their drug-resistance profiles and possible effects on treatment outcome.
2	Title (in Japanese)	ベトナムにおける結核症に関する研究 結核菌北京型株の蔓延と多剤耐性に関わる研究
3	Main researcher	Naoto Keicho (NCGM/Research Institute of Tuberculosis, JATA) Luu Thi Lien (Hanoi Department of Health) Pham Huu Thuong (Hanoi Lung Hospital)
4	Co-Researcher(s)	Vu Cao Cuong (Hanoi Department of Health) Nguyen Phuong Hoang (Hanoi Lung Hospital) Nguyen Van Hung (National Lung Hospital) Shinji Maeda (Research Institute of Tuberculosis, JATA) Minako Hijikata (NCGM/Research Institute of Tuberculosis, JATA) Nguyen Thi Le Hang (NCGM-BMH Medical Collaboration Center) Shinsaku Sakurada (International Bureau, NCGM)
5	Resource of fund	the Program of Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), MEXT
6	Affiliation(s) in Viet Nam	Hanoi Lung Hospital (HLH), Viet Nam National Lung Hospital (NLH), Viet Nam
7	Period of the research	2010-2015
8	Publications	1. Matsushita I, Hang NT, Hong le T, Tam do B, Lien LT, Thuong PH, Cuong VC, Hijikata M, Kobayashi N, Sakurada S, Higuchi K, Harada N, Keicho N. **Dynamics of immune parameters during the treatment of active tuberculosis showing negative interferon gamma response at the time of diagnosis.** Int J Infect Dis. 2015 ;40:39-44. 2. Hang NT, Maeda S, Keicho N, Thuong PH, Endo H. **Sublineages of *Mycobacterium tuberculosis* Beijing genotype strains and unfavorable outcomes of anti-tuberculosis treatment.** Tuberculosis (Edinb). 2015 ;95:336-42. 3. Hijikata M, Matsushita I, Hang NTL, Thuong PH, Sakurada S, Cuong VC, Lien LT, Keicho N. **Dual-specificity phosphatase 14 gene polymorphism in Viet Namese patients with pulmonary tuberculosis.** European Respiratory Society International Congress 2015; Amsterdam, Netherlands, September 26-30, 2015
9	Summary: **Overall purpose** • To strengthen collaborative research work on tuberculosis (TB) between Viet Nam and Japan. • To prevent generation and spread of drug-resistant TB and TB-HIV co-infection.	

	Output A. NCGM-RIT-HLH collaboration 1. Analysis of Hanoi-TB data containing clinical, genome-epidemiological, immunological and bacteriological information, and specimens. 2. Improvement of diagnosis, monitoring, treatment and prevention of TB and understanding process of TB infection and development. 3. Identification and reduction of risk factors to prevent spread of drug-resistant TB and TB-HIV co-infection. 4. Identification of possible risk factors to unfavorable anti-TB treatment outcomes B. NCGM-RIT-NLH collaboration 1. Genome epidemiology of *Mycobacterium tuberculosis* (MTB) strains in Hanoi. 2. Analysis of drug-resistant MTB. 3. Analysis of reactivation and re-infection of MTB after anti-TB treatment.

Research No.4

1	Title (in English)	Support for Strengthening Medical Treatment Ability of the Childhood Cancer in Viet Nam
2	Title (in Japanese)	ベトナムにおける小児がん医療の診療能力強化を目的とした支援
3	Main researcher	Hiroyuki Shichino (Director of pediatrics, national Center For Global Health And Medicine)
4	Co-Researcher(s)	Noriko Sato, Junko Yamanaka, Hideko Uryu, Mizue Tanaka, Hiroki Kato, Motohiro Matsui
5	Resource of fund	International Promotion of Japan's Healthcare Technologies and Services, NCGM Program International Health Research (A27-5) from Ministry of Health Labor and Welfare of Japan
6	Affiliation(s) in Viet Nam	National Hue Central Hospital (Hue)
7	Period of the research	April 2015- March 2016
8	Publications	
9	Summary:	

BACKGROUND: Eighty percents of world childhood cancer patients are children in the developing countries. There are many problems such as misdiagnoses, delay of discoveries, lack of offer of treatment. Many childhood cancer patients were supposed to be untreated, and there were not the grasp of accurate number of the childhood cancer patients. And there were small numbers of specialists of pediatric cancer.

PURPOSE: To support for strengthening diagnosis, medical treatment, supportive care abilities of the childhood cancer in the pediatrics and pediatric surgery of leading hospitals in Viet Nam.

METHODS: Sending experts well-versed in childhood cancer, to provide training in the field of childhood cancer diagnosis, treatment, supportive care. Accepting healthcare providers from Viet Nam as trainees for studying childhood cancer.

RESULTS: We sent total 14 Japanese experts to Hue central hospital in two times. And accepted 10 healthcare providers from Hue to Japan in one time. And also in August we held an educational seminar about childhood cancer .We had total 140 attendants in this seminar.

CONCLUSION: We could support to improve the medical treatment ability of the staff concerned with childhood cancer about such as a diagnosis, treatment, nursing care, supportive care. And also we thought we could increase the number of childhood cancer patients who had been diagnosed and treated step by step.

Research No.5

1	Title (in English)	Improving the quality of medical treatment for childhood cancer in developing countries
2	Title (in Japanese)	開発途上国の小児がんの診療レベルの向上に関する研究
3	Main researcher	Junko Yamanaka (Staff of Pediatrics Division, National Center For Global Health And Medicine)
4	Co-Researcher(s)	Hiroyuki Shichino, Noriko Sato, Takeji Matsushita
5	Resource of fund	International Health Research and Development Fund (25-13)
6	Affiliation(s) in Viet Nam	National Hue Central Hospital (Hue)
7	Period of the research	April 2013- March 2016
8	Publications	
9	Summary:	

BACKGROUND: In developing countries, major causes of child mortality include neonatal disease, congenital anomaly, infection, accidental death, as well as childhood cancer. The medical aid programs of children's cancer has been given less attention though recently Western industrialized countries have actively begun to support children suffering from cancer in developing countries.

PURPOSE: The aim of this study is to achieve better medical quality of childhood cancer by improving diagnostic skills, supportive care, and treatment environments in Viet Nam. Having developed the basic strategy for this project, we are considering implementing our activities in other Southeast Asian nations that may require support in the field of children's cancer treatment.

METHODS AND RESULTS: During the first year of our cooperative study with Viet Nam, we investigated the status of children's cancer treatment at National Hue Central Hospital, pointed out problems and concerns, and discussed them with the local physicians. We have focused on children's acute lymphoblastic leukemia (ALL), which is the most common oncology disease there. They have treated over 100 ALL cases since 2007, with 20-30 new ALL patients per year, following a modified protocol with lower-toxicity. Supportive care – such as oral care, administration of injection rote, prevention of infection, and blood transfusion – may have room for improvement. Patient families education is also important to reduce abandonment of treatment from urban areas. During our second year of study, we investigated further into various aspects that influence treatment outcomes. Overall, children's ALL treatment in Hue has been improving since they began to treat according to unified protocols, although treatment outcomes have not reached those of advanced countries. The approximate rate of the hematology malignancy patient's event-free survival is 50%; half of patients failed during treatment. Therefore we have decided to conduct a review of these deaths in order to better understand the problems with children's ALL treatment in Hue. During our final year we will collect and analyze the data and summarize the outcomes of this deaths reviews, which we hope will lead to the improved treatment of childhood cancer in Viet Nam.

CONCLUSION: It is important initially to establish a registration system of disease and look into events (deaths) in order to grasp and analyze the current situation and treatment outcomes, and then establish standards for treatment management on a per-country basis.

Research No.6

1	Title (in English)	Impact of a life style intervention in incident and prevalence of overweight and obesity among secondary school children in Hanoi
2	Title (in Japanese)	ハノイ市の中学生における肥満・過体重に対する生活習慣介入に関する研究
3	Main researcher	Kajio H (Director, Department of Diabetes, Endocrinology and Metabolism, NCGM, Japan) Anh NQ (Director, BMH, Viet Nam) Lien DTK (Director, Department of Diabetes and Endocrinology, BMH, Viet Nam)
4	Co-Researcher(s)	JAPAN: Matsushita Y (NCGM), Tsujimoto T(NCGM), Hara M (Tokyo Metropolitan Hiroo General Hospital) Viet Nam: Thanh DVT (BMH), Thanh NTT (BMH), Thuy PTP (MCC)
5	Resource of fund	1) The Grant of National Center for Global Health and Medicine, NCGM, Japan 2) The Grant of Ministry of Health, Labor and Welfare of Japan
6	Affiliation(s) in Viet Nam	Bach Mai Hospital
7	Period of the research	Dec 2012 -
8	Publications	In Preparation
9	Summary: Overweight and obesity lead to adverse metabolic effects on blood pressure, cholesterol, triglycerides and insulin resistance. Risks of coronary heart disease, ischemic stroke and type 2 diabetes mellitus increase steadily with increasing body mass index (BMI). Overweight and obese children are likely to stay obese into adulthood and more likely to develop non-communicable diseases (NCD) like diabetes and cardiovascular diseases at a younger age. In the developing countries, nowadays, the increasing prevalence and incidence of overweight and obesity is a serious public health problem following the social and economic development of the country. The prevalence has increased at an alarming rate even in children. Our aim of this study is to study the impact of a life style intervention in incident and prevalence of overweight and obesity among secondary school children in Hanoi from 2012 to 2015. **Goal of the theme:** Overall goal: Reduce prevalence and incident in secondary school children. **Specific goals:** 1) To find whether length of education had an impact on prevalence and incident of overweight and obesity in the children. 2) To evaluate the impact of the intervention to lifestyle changes in dietary control, physical exercise among the children. 3) To improve the knowledge of overweight and obesity and prevention against lifestyle-related diseases in the secondary school children. **METHODS:** We started an intervention study with controlled group to see longitudinal changes of anthropometrical indices, diet and intergraded physical activities, lifestyle and biomarkers such as FBG, HbA1c, insulin level, lipid profile, and adiponectin after the baseline surveillance in Dec, 2013 and in Jan, 2014.	

We recruited 821 children of 6th grade from 4 different schools (Cat Linh school, Nguyen Cong Tru School, Phan Chu Trinh School, Dong Da school), which were randomly selected from 2 urban districts in Hanoi. The information of the baseline surveillance included the lifestyle conditions and the knowledge levels of the children as well as the parents provided by the questionnaires. After allocation of 4 schools into two groups, two schools for the intervention group and the other two schools for the control group, we have been performing intervention activities. The intervention activities are integrated by four components; physical activity, nutritional, lifestyle behavior change and wide communication tools. We provided the participants with the pedometers, and the participants in the intervention group were also provided with the scales. As for the behavior intervention, we set up several targets to promote the participants in the intervention group to continue the activities with self-monitoring, goal setting, and problem solving.
The 1-year mid-term surveillance was performed in Dec, 2014 and in Jan, 2015. The 2-year final surveillance was performed in Jan, 2016. The analysis of the results is now being performed.

RESULTS: We obtained the baseline data from 821 children of 4 schools. We found that the prevalences of children with underweight, normal-weight, overweight and obesity were 4.1%, 59.7%, 16.9% and 19.2%, respectively, following WHO standard cut-offs. Multiple logistic regression analysis to predict risk factors for overweight/obesity (OW/OB) after adjustment with sex, the OR of OW/OB for children is increased by paternal OW/OB (reference: BMI 18.5-24.9 kg/m2; BMI≥25: 2.02, p=0.001), maternal OW/OB (reference: BMI 18.5-24.9 kg/m2; BMI≥25: 2.831, p=0.001), parental OV/OB (reference: parental no OW/OB, father or mother OW/OB: 2.22, $p < 0.001$; both of parental OW/OB: 6.59, $p = 0.024$), increased birth weight (reference: 2500-3500g; ≥ 3500g: 1.52, $p = 0.02$), less sleeping hour per day (reference: < 8 hours; 8 -11: 0.57, $p < 0.001$; >11:0.44, $p = 0.02$), less physical activities to lose weight (reference: no, yes : 6.185, $p < 0.001$), less food to lose weight (reference: no, yes : 8,239, $p < 0.001$), and more vegetable to lose weight (reference: no, yes : 3.908, $p < 0.001$). At the 2-year final surveillance performed in Jan, 2016, the data of the questionnaires were collected from 739 students and 660 parents. The health check was performed for 731 students. The laboratory data were collected for 702 students. The data for the 2-year surveillance is now under analysis.

DISCUSSIONS AND CONCLUSIONS: At the beginning of the study, we identified the higher prevalence of overweight or obesity in school children, and clarified the importance factors influencing on the appearance of overweight or obesity. These factors include family anthropometrical factors as well as several lifestyle factors such as sleeping duration, food and physical activities. We finished the intervention trial. After the analysis of the data, we will identify how much the behaviors of the students and their parents changed through the intervention and how we should make the intervention to change the lifestyle for the children and their family to reduce the prevalence and incident of overweight and obesity in the children.

Research No.7

1	Title (in English)	Assessment of the interface between malaria control program and health system strengthening
2	Title (in Japanese)	マラリア対策とヘルスシステム強化に関する研究
3	Main researcher	Hiroshi Ohara
4	Co-Researcher(s)	Vu Huy Nam (Dept. of Planning, National Institute of Malariology, Parasitology and Entomology) Pham Thi Thanh Thuy (Dept. of Infectious Diseases, Bach Mai Hospital) Jeevan B. Sherchand (Dept. of Public Health, Institute of Medicine, Tribhuvan University, Nepal)
5	Resource of fund	Grants of National Center for Global Health and Medicine (27-4, 24-5)
6	Affiliation(s) in Viet Nam	National Institute of Malariology, Parasitology and Entomology (NIMPE), Bach Mai Hospital
7	Period of the research	October 2014- March 2017
8	Publications	Ohara H, Sherchand JB, Vu HN, et al. Assessment of health systems in relation to interface between malaria control programs and health system strengthening: comparative study between Nepal and Viet Nam. J Inst Med, 37(1): 11-20, 2015. Ohara H, Vu HN, Sherchand JB, et al. Assessment of malaria control programs in relation to general health systems with special reference to equity in bed net use. NCGM report, submitted to WPRO/WHO available at: http://ncgmimcj.ecnet.jp/HP/library/research-_doc/ncgm_report_oct 2013.pdf
9	Summary: Malaria has been a high priority issue in many tropical and sub-tropical countries. In order to implement malaria control program effectively, it is crucial to utilize health system effectively. In this study, interactions between malaria control program and health system strengthening was assessed. The studies were conducted in Viet Nam and Nepal with the methods of key informant interviews, investigation in malaria endemic areas and document review. As retrospective study, encountered challenges in malaria control and interventions for them were analyzed from the viewpoint of interactions between disease specific program and general health system using the 6 Building Blocks of Health System Strengthening of WHO (Leadership and Governance, Service delivery, Workforce, Information system, Medical products and technologies, and Financing). In addition, current challenges in malaria control were identified and possible interventions were discussed. In Viet Nam, leading good practices included: 1) Strong government commitment for malaria control, 2) National strategy for rural development and intensified education for residents, 3) Effective vertical system from national to village level for malaria surveillance and service delivery, 4) Domestic antimalarial production and high coverage of control measures, 5) Strengthening the capacity of health workers along with mobilization of mass organizations, and 6) Support from international organizations. In Nepal, malaria was showing high morbidity and mortality rate until the middle of 1990s, however thereafter it decreased remarkably due to the effective control program. Leading factors contributed to the successful control were identified as the best practices. The followings are leading current challenges in malaria control in Viet Nam: 1) Increase of malaria in some areas associated	

	with population movement, 2) Shortage of health manpower in remote areas, 3) Poorly developed reporting system from the private health sector, 4) Difficulty in treatment due to increasing resistance of *P. falciparum* to anti- malaria drugs, 5) Low incentive for health workers. In both countries (particularly in Viet Nam), it was recognized that the malaria control program contributed to strengthening of general health system and thus strengthened general health system contributed to the smooth implementation of other health programs (synergic effect). Effective implementation under the strong leadership of the governments utilizing the existing health system was outstanding in both countries. Besides, strengthening of the vertical health program appeared to have a good impact on the general health system, particularly at the primary level.

Research No.8

1	Title (in English)	A study on sinopulmonary disease (chronic rhinosinusitis with chronic lower airway infection) in Viet Nam
2	Title (in Japanese)	ベトナムにおける副鼻腔気管支症候群の研究 (25 指 5 の分担研究として)
3	Main researcher	Naoto Keicho (NCGM/Research Institute of Tuberculosis, JATA) Ngo Quy Chau (Bach Mai Hospital) Le Cong Dinh (Bach Mai Hospital)
4	Co-Researcher(s)	Minako Hijikata (NCGM/Research Institute of Tuberculosis, JATA) Yuichi Majima (Ise Municipal General Hospital) Takuro Shimbo (NCGM) Pham Minh Thong (Bach Mai Hospital) Pham Thien Ngoc (Bach Mai Hospital) Phan Thu Phuong (Bach Mai Hospital) Le Thi Tram (Bach Mai Hospital) Nguyen Thi Le Hang (NCGM-BMH Medical Collaboration Center) Nguyen Thu Huyen (NCGM-BMH Medical Collaboration Center)
5	Resource of fund	a grant of National Center for Global Health and Medicine
6	Affiliation(s) in Viet Nam	Bach Mai hospital
7	Period of the research	2013-2015
8	Publications	Ngo Quy Chau, Le Cong Dinh, Phan Thu Phuong, Nguyen Thi Le Hang, Pham Minh Thong, Nguyen Thu Huyen, Minako Hijikata, Ikumi Matsushita, Naoto Keicho. **Characterization of patients with sinopulmonary disease in a Viet Namese hospital.** Presented at the 55th Annual Meeting of the Japanese Respiratory Society 2015, Tokyo, Japan.
9	Summary: **Overall purpose** • To identify host genetic factors involved in development of sinopulmonary disease in Viet Nam. • To characterize clinical background of sinopulmonary disease in Viet Nam. • To investigate prevalence and risk factors for chronic lower respiratory infection among patients with chronic rhinosinusitis in Viet Nam.	

Research No.9

1	Title (in English)	Research on establishing basis of international collaborative studies in Viet Nam
2	Title (in Japanese)	ベトナム海外拠点における高品質な臨床疫学研究の実施と支援体制の整備に関する研究　(25 指 5 の分担研究として)
3	Main researcher	Naoto Keicho (NCGM/Research Institute of Tuberculosis, JATA)
4	Co-Researcher(s)	Hoang Van Minh (Hanoi Medical University)
5	Resource of fund	a grant of National Center for Global Health and Medicine
6	Affiliation(s) in Viet Nam	NCGM-BMH Medical Collaboration Center
7	Period of the research	2013-2015
8	Publications	
9	Summary: **Overall purpose** To strengthen skills of collaborative research in NCGM-BMH Medical Collaboration Center Activities 1. Lecture training on skills of implementation of clinical research 2. On the job training as research associates for model research	

Research No.10

1	Title (in English)	Chronological, geographical, and seasonal trends of human cases of avian influenza A (H5N1) in Viet Nam, 2003–2014: a spatial analysis
2	Title (in Japanese)	ベトナムにおける 2003-2014 における鳥インフルエンザ A（H5N1）のヒト感染例の時間・空間・シーズン的傾向：空間分析
3	Main researcher	Toshie Manabe, Ngo Quy Chau, and Koichiro Kudo
4	Co-Researcher(s)	Kazue Yamaoka, Shinyu Izumi, Jin Takasaki, Toshiro Tango, Nguyen Gia Binh
5	Resource of fund	Japan Initiative for Global Research Network on Infectious Disease: J-GRID
6	Affiliation(s) in Viet Nam	Bach Mai Hospital
7	Period of the research	2010-2014
8	Publications	Manabe et al. BMC Infectious Diseases (2016) 16:64 (DOI 10.1186/s12879-016-1391-8)
9	Summary:	

BACKGROUND: Human cases of highly pathogenic avian influenza A (H5N1) virus infection continue to occur in Southeast Asia. The objective of this study was to identify when and where human H5N1 cases have occurred in Viet Nam and how the situation has changed from the beginning of the H5N1 outbreaks in 2003 through 2014, to assist with implementing methods of targeted disease management.

METHODS: We assessed the disease clustering and seasonal variation of human H5N1 cases in Viet Nam to evaluate the geographical and monthly timing trends. The clustering of H5N1 cases and associated mortality were examined over three time periods: the outbreak period (2003–2005), the post-outbreak (2006–2009), and the recent period (2010–2014) using the flexibly shaped space-time scan statistic. The most likely cases to co-cluster and the elevated risks for incidence and mortality were assessed via calculation of the relative risk (RR). The H5N1 case seasonal variation was analysed as the cyclic trend in incidence data using Roger's statistical test.

RESULTS: Between 2003 and 2005, H5N1 cases (RR: 2.15, $p = 0.001$) and mortality (RR: 2.49, $p = 0.021$) were significantly clustered in northern Viet Nam. After 2010, H5N1 cases tended to occur on the border with Cambodia in the south, while H5N1 mortality clustered significantly in the Mekong delta area (RR: 6.62, $p = 0.002$). A significant seasonal variation was observed ($p < 0.001$), with a higher incidence of morbidity in December through April.

CONCLUSIONS: These findings indicate that clinical preparedness for H5N1 in Viet Nam needs to be strengthened in southern Viet Nam in December–April.

Research No.11

1	Title (in English)	International collaborative study for diagnosis of avian influenza virus infection and other respiratory viral infections
2	Title (in Japanese)	鳥インフルエンザウイルス感染症と他の呼吸器系ウイルス感染症の診断に関する国際共同研究
3	Main researcher	Prof. Nguyen Gia Binh, Bach Mai Hospital Vu Thi Tuong Van, Bach Mai Hospital Noriko Nakajima, NIID, Japan Tsutomu Kageyama, NIID, Japan Jin Takasaki, DCC, Respiratory Medicine, NCGM, Japan
4	Co-Researcher(s)	Viet Nam: Dao Xuan Co, Nguyen Dang Tuan (ICU), Truong Thai Phuong, Le Thi Ngan (Microbiology), Do Van Thanh (International and ID Dept.), Pham Phuong Thuy (MCC), Do Duy Cuong (Infectious disease), Phan Thu Phuong – Respiratory Center JAPAN: Ikuyo Takayama(Center for Influenza Virus Research, NIID), Shoji Kawachi (Dept. of Anesthesiology, NCGM), Naoyuki Hirata (Dept. of Anesthesiology, Sapporo medical university), Tamano Matsui, Yuzo Arima (Infectious Diseases Surveillance Center, NIID), Tadaki Suzuki (Dept. of Pathology, NIID)
5	Resource of fund	Japan Agency for Medical Research and Development, AMED
6	Affiliation(s) in Viet Nam	Bach Mai Hospital
7	Period of the research	September 2015 to March 2016
8	Publications	None
9	Summary:	

Project objectives

1. Introduction of the bed-side detection system for influenza viruses and other respiratory virus genomes for the rapid diagnosis and effective treatment.
2. The assessment of the sensitivity and specificity of this rapid detection system in comparison with the results using real-time RT-PCR/PCR system.

METHOD: A Preliminary study for 100 cases with severe respiratory infectious diseases in Bach Mai Hospital. This study will take place at the intensive care unit (ICU), department of respiratory medicine, and department of infectious disease in Bach Mai Hospital, Hanoi, Viet Nam.

The study subjects are hospitalized patients with respiratory infectious diseases in ICU, respiratory department and infectious disease department in Bach Mai Hospital, Hanoi, Viet Nam from September 2015 to March 2016 as 1st phase. The comparison between the results of RT-LAMP and real time PCR method and the evaluation of RT-LAMP bed-side system in specificity, in sensitivity, in convenience, and in rapidity.

This direct RT-LAMP assay kit can detect FluA, FluB, FluC, H1pdm09, H3, H5, H7, MERS-CoV, CoV-HKU1, CoV-NL63, CoV-OC43, CoV-229E, hPIV1, hPIV2 and hPIV3.

The comparison between the results of RT-LAMP and real time PCR method and the evaluation of “direct RT-LAMP bed-side system” in specificity, in sensitivity, in convenience, and in rapidity.

RESUTS: Total 120 samples are collected as of Mar. 10th, 2016. Of 109 which have already analyzed, 43 were positive with RT-LAMP, 11 of 44 from Respiratory Center, 18 of 48 from ICU, and 14 of 17 from Infectious Disease Department. Of 43 positive cases, 33cases were positive for influenza A (15 with H1N1pdm, and 13 with H3), 4 with influenza B, 6 with Coronavirus, and 1with parainfluenza virus. Realtime PCR was positive in 81 cases. For influenza, the results were consistent by each other.

CONCLUSION: RT-LAMP method for detecting influenza was as reliable as RT-PCR. In the next stage, this method will be applied as a portable genetic rapid diagnostic system which can be performed by clinicians in bed-side even in rural areas where H5N1 outbreak occurs.

2. The International Promotion of Japan's Healthcare Technologies and Services in 2015

This program has been commissioned by the Ministry of Health, Labour and Welfare Japan since fiscal 2015. The purpose is to extend Japanese healthcare and services as well as experiences on health systems to the world. Areas of the program include (1) Japanese health technologies, medical devices, and medicines, (2) management of health facilities, (3) health regulation, medical insurance, medical environment management, (4) health information systems, and (5) global health issues such as emerging and re-emerging infectious diseases, an aging society, maternal and child health, nutrition, non-infectious diseases, and disaster response. The program consists of two methods; dispatch of Japanese specialists and acceptance of foreign trainees in Japan.

The seven programs were carried out in Viet Nam by NCGM as follows:

- Support for Strengthening Medical Treatment Ability of the Childhood Cancer, Viet Nam
- Strengthening health Staffs' Capacity of Quality Management in Healthcare, Viet Nam
- Human resource development in facilities under cooperation agreement in Viet Nam
- The project for strengthening Clinical Laboratory,
- Radiology and Pharmaceutical departments at the Hospitals
- Policy dialogue toward UHC/health social security
- Strengthening Human Resource Development for Nursing and Midwifery in South East Asia

3. Other activities, topics

International Nursing Practicum for Nursing Students at the National College of Nursing, Japan

We conducted a one-week nursing practicum in Viet Nam as part of the compulsory subject of International Nursing Practicum for fourth-year undergraduate students in collaboration with Hai Duong Medical Technical University (HMTU), Viet Nam.

The International Nursing Practicum is designed to enhance students' abilities to understand the current situation of nursing and health care practice in developing countries, whereby promoting the development of nursing theory with international perspectives to facilitate international health cooperation in nursing. As a prerequisite, students are required to complete the international nursing theory course.

One hundred students were divided into 14 groups, and each group was assigned several presentation topics to work toward the goals of the practicum. Before departing for Hai Duong, Viet Nam, where the practicum took place, students rehearsed their presentation in English in order to improve the quality of presentation and share their knowledge among groups in preparation for the practicum.

On the first day of the practicum, students gave their presentation in front of the faculty members and undergraduate students at HMTU and NCNJ. They then visited several institutions in Hai Duong province, such as provincial hospital, district hospital, specialty hospital, leprosy village, social welfare institution, and community health center.

On the last day of the practicum, each group presented the summary of students' experiences at HMTU. Back in Japan at NCNJ, a poster presentation was held in the entrance hall, which gave students an opportunity to summarize what they had learned through the practicum in both Japan and Viet Nam, as well as to inform other junior students and faculty members of their valuable experiences.

Student evaluation revealed that most students wished to contribute what they had learned to nursing activities in Japan and promotion of international health cooperation.

Sister Renal Center Program officially approved by International Society of Nephrology (ISN)

Department of Nephrology, NCGM,

* Emerging Center: Department of Nephro-Urology, Bach Mai Hospital (BMH) in Hanoi, Viet Nam
* Supporting Center: Department of Nephrology, NCGM, Shinjuku-ku, Tokyo, Japan

[Project Concept]

First of all, the ISN Sister Renal Center (SRC) Program (SRCP) helps improve how nephrology is practiced in emerging countries by linking emerging renal centers or units with established centers of excellence in the developed world. Department of Nephrology, NCGM, decided to clinically and technically assist and support Department of Nephro-Urology, BMH for SRCP in 2013. Fortunately, our program was officially approved by ISN in early 2014, and our collaborative project has been going on since then.

BMH is one of the largest, leading hospitals in Northern Viet Nam, focusing on Internal Medicine and medical education, in cooperation with Hanoi Medical University. All of the BMH nephrologists are well educated and have a wide-ranging experience with various projects. On the other hand, the Bureau of International Medical Cooperation, Japan (IMCJ) is a semi-national institute founded in 1986, belonging to NCGM in Japan. It has been functioning as a leading Japanese international cooperation agency in the health sector in association with the Ministry of Health, Labour and Welfare, the Ministry of Foreign Affairs, Japan International Cooperation Agency (JICA) and the World Health Organization (WHO). IMCJ and some of the clinical departments of NCGM have continuously supported BMH to build up a modern medical system and to introduce up-to-date clinical skills. However, there has recently been no collaboration in nephrology between NCGM and BMH. Certainly our SRCP will further raise the capacity of the Department of Nephro-Urology at BMH to instruct many nephrologists working at other local and smaller hospitals in Northern Viet Nam and consequently contribute to the further development at those institutions. In addition to the Department of Nephrology of NCGM, IMCJ which has participated in many international cooperative projects would gladly support the Department of Nephro-Urology at BMH and its nephrologists. Based on SRCP, we believe that both departments from NCGM and BMH can collaboratively start some clinical study in nephrology, resulting in the progress of clinical nephrology and medical care in Viet Nam.

Research

1	Title (in English)	Research on Improvement of CKD and Dialysis Management in Hanoi, Viet Nam
2	Title (in Japanese)	ベトナム国ハノイにおける慢性腎臓病管理・透析の調査と質の向上に関する研究
3	Main researcher	Fumihiko Hinoshita, (MD, Ph.D, Head, Department of Nephrology, NCGM)
4	Co-Researcher(s)	Manami Tada (Department of Nephrology, NCGM)NCGM(26-3)
5	Resource of fund	NCGM(26-3) SRCP funded by International Society of Nephrology
6	Affiliation(s) in Viet Nam	Bach Mai Hospital
7	Period of the research	January 2014 – December 2017
8	Publications	None
9	Summary:	

Overall purpose

- To establish further collaboration between Dept of Nephrology, NCGM and Dept of Nephro-Urology, BMH under the Sister Renal Center Program officially approved by International Society of Nephrology (ISN)
- To establish a sophisticated means of treating patients with CKD in the preservation period and retard the progression of CKD in those patients in BMH as well as local hospitals in Northern Viet Nam
- To improve management of hemodialysis and other blood purifications at BMH and the dialysis units in Hanoi
- To conduct a survey of DM nephropathy in Hanoi

Activities

- A nephrologist and a medical engineer from NCGM had a lecture on CKD management and hemodialysis, especially management of HD water at BMH.
- Two nephrologist and a nurse from BMH had a training at NCGM.
- We started a survey on water hardness and residual chloride of HD water to improve quality of HD at BMH and HD units in Hanoi.
- Upgrading from Level C to Level B under the Sister Renal Center Program was officially approved by ISN in January, 2016 according to the good evaluation of the activities between NCGM and BMH.

III. Reference

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EPIDEMIOLOGY

Sublineages of *Mycobacterium tuberculosis* Beijing genotype strains and unfavorable outcomes of anti-tuberculosis treatment

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SUMMARY

The influence of *Mycobacterium tuberculosis* (MTB) lineages/sublineages on unfavorable tuberculosis (TB) treatment outcomes is poorly understood. We investigated the effects of Beijing genotype sublineages and other factors contributing to treatment outcome. Patients newly diagnosed with sputum smear-positive and culture-positive TB in Hanoi, Vietnam, participated in the study. After receiving anti-TB treatment, they were intensively followed up for the next 16 months. MTB isolates collected before treatment were subjected to drug susceptibility testing, and further analyzed to determine MTB (sub) lineages and their clonal similarities. Of 430 patients, 17 had treatment failure and 30 had TB recurrence. Rifampicin resistance was associated with treatment failure {adjusted odds ratio = 6.64 [95% confidence interval (CI), 1.48–29.73]}. The modern Beijing genotype was significantly associated with recurrent TB within 16 months [adjusted hazard ratio = 3.29 (95% CI, 1.17–9.27)], particularly after adjustment for the relevant antibiotic resistance. Human immunodeficiency virus coinfection and severity on chest radiographs were not significantly associated with unfavorable outcomes. Our findings provide further understanding of the influence of MTB strains on unfavorable treatment outcomes. Multiple risk factors should be considered for the optimal management of TB.

1. Introduction

Tuberculosis (TB) is a chronic infection that continues to be a major public health problem worldwide, with 8.6 million new cases and 1.3 million deaths in 2012 [1]. Unfavorable treatment outcome, including treatment failure and recurrence, is a major risk factor for drug resistance in TB [2], which increases the TB burden [3]. Recurrence is defined as an active TB episode that reoccurs after initial successful treatment. According to the World Health Organization (WHO) Report 2013 [1], of the 6.1 million cases of TB that were identified, 0.3 million had recurrent episodes (including exogenous reinfection and endogenous reactivation) after being previously cured.

Previous studies have evaluated risk factors for TB recurrence, which include severity of disease indicated on chest radiographs (e.g., the presence of cavitation and the extent of pulmonary involvement) [4,5], drug resistance [6], microbial load at diagnosis, or human immunodeficiency virus (HIV) coinfection [4]. Because recurrence occurs as a result of dynamic interactions between host and pathogen [7], the *Mycobacterium tuberculosis* (MTB) genotype should also be considered a potential risk factor for recurrence. The MTB Beijing genotype strains account for the majority of the East Asian lineage, one of the seven major MTB lineages in the world [8], and are becoming widespread even outside Asia [9]. These strains appear to be associated with an increased risk of TB recurrence, according to several reports [10–13].

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The Beijing genotype strains belong to one of the two major sublineages, ancient (atypical) and modern (typical) types, based on the absence or presence of an IS*6110* insertion in a particular chromosomal position designated as the NTF region of the MTB genome [14]. Molecular epidemiological studies have characterized possible differences in phenotypes between the sublineages: strains of the modern Beijing sublineage are adapted to spread and cause disease more easily than those of the ancient sublineage in different regions of the world [15,16], whereas the ancient Beijing sublineage is often associated with drug resistance, including multidrug resistance (MDR) or extremely drug-resistant TB [17], pyrazinamide (PZA or Z) or rifampicin (RMP or R) resistance [18], and isoniazid (INH or H) or streptomycin (SM or S) resistance [19]. However, associations between Beijing sublineages and unfavorable treatment outcomes have not yet been fully investigated.

Vietnam is a Southeast Asian country stretching over 1,800 km from north to south. It is one of 22 countries with a high TB prevalence worldwide (218 per 100,000 in 2012) [1]. Although the treatment success rate for new cases was reported to be between 85% and 93% from 1995 to 2011 [1], more than 7200 cases were identified as recurrent TB in 2012 [1]. Regional differences in MTB genotypes have been observed. In southern Vietnam, the modern sublineage is reportedly predominant over the ancient sublineage [20]; and the Beijing genotype, as a whole, has been reported as a risk factor for TB recurrence, when compared with non-Beijing genotypes [13]. In Hanoi, in northern Vietnam, the ancient sublineage (37.5%) is more prevalent than the modern sublineage (20.9%) [19]. In the present study, we investigated whether these two Beijing sublineages are similarly associated with treatment failure or recurrence in newly diagnosed active pulmonary TB patients in Hanoi.

2. Materials and methods

2.1. Ethics statement

Written informed consent was obtained from each participant; parents provided written informed consent for minors. This study was approved by the ethical committees of the Ministry of Health of Vietnam, the National Center for Global Health and Medicine, and the Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Japan.

2.2. Study sites, recruitment of patients, and sample collection

Patients were recruited within part of a cohort study reported elsewhere [21]. Residential areas of the patients were divided into three categories based mainly on population density, year of establishment, and urbanization speed: suburban (1500–2500 individuals/km^2), old urban (25,000–26,000 individuals/km^2), and new urban (2800–5300 individuals/km^2) areas [22]. In summary, patients over 16 years old residing in the Hanoi area who suffered from newly diagnosed smear-positive pulmonary TB and agreed to participate in the study were recruited from July 2007 to March 2009. These patients were interviewed by pretrained healthcare staff using a structured questionnaire. Before initiation of treatment, sputum specimens and blood samples were collected. Patients then received the standard 8-month regimen that was commonly administered during the study period in Vietnam: INH, RMP, PZA, and SM or ethambutol (EMB or E) for 2 months followed by INH and EMB for 6 months [2S(E)HRZ/6HE]. Drug susceptibility testing was performed retrospectively on the MTB isolates, which were stored in a freezer before anti-TB treatment began. Thus, susceptibility test results were not available while deciding treatment schedules, and the standard regimen was followed according to the national TB control program guidelines at the time of the study.

During treatment, culture tests were repeated when smear tests were confirmed positive at 2, 5, or 7 months. During the 16-month post-treatment follow-up, sputum smear and culture tests were scheduled at 2, 4, 7, 10, and 16 months for all enrolled cases.

2.3. Identification of MTB, drug susceptibility testing and molecular genotyping

MTB, drug susceptibility, and molecular genotypes were identified as previously reported [19,22]. In short, the niacin test was initially used for MTB identification, and drug susceptibility testing was performed for INH, SM, RMP, and EMB on the basis of the proportional method recommended by WHO. Beijing and non-Beijing strains were distinguished by a single-nucleotide polymorphism (SNP) at position 779,615 [23] and spoligotyping results [24]. Their genotypes were identified using the international MTB database (SpolDB4) [25]. Ancient and modern Beijing genotypes were further distinguished using the polymerase chain reaction method [26]. Variable number of tandem repeat (VNTR) analysis was performed using the international standard 24 mycobacterial interspersed repetitive unit-VNTR system [27] with four additional loci recommended for the Beijing genotype strains [28]. Genetic clustering was defined by a complete match of the VNTR profile of the 28 loci.

2.4. Definitions of treatment failure and TB recurrence

Treatment failure and TB recurrence were defined on the basis of the WHO Global Tuberculosis Report 2013 [1]. In summary, treatment failure was noted when the smear and culture were positive at ≥5 months or when the smear was positive but culture was not performed, clinical and/or chest radiography findings indicated failure, and the category switched to the regimen for retreatment. Recurrence was noted when patients were cured after treatment and then suffered a second TB episode. The second episode was confirmed if the sputum culture was positive at the time of recurrence, or if a culture result was not available or was difficult to assess (<5 colonies) [29], but the smear was positive in patients with clinical and/or chest radiographic abnormalities indicating the necessity of retreatment. After the expert committee members' review in Vietnam, the category II regimen (2SHRZE/1HRZE/5$H_3R_3E_3$) for retreatment was started in all patients with treatment failure or TB recurrence.

2.5. Statistical analysis

Logistic regression models were used to investigate factors possibly associated with treatment failure, and adjusted odds ratios (aORs) and 95% confidence intervals (CIs) were calculated. Using a logistic regression model, treatment failure was set as a binominal outcome variable, and the explanatory variables were MTB lineages/sublineages and relevant antibiotic resistance. The log-rank test for equality across strata was also used to investigate the association between MTB lineages and time to recurrence. After testing the proportional hazard assumption, Cox models were used to assess multiple risk factors for recurrence, similar to the logistic regression models. Adjusted hazard ratios (aHRs) and 95% CIs were calculated. Statistical analysis was performed using Stata version 12 (StataCorp, College Station, TX, USA), and P values of <0.05 were considered statistically significant.

3. Results

3.1. Characteristics of the study population

In the present study, 489 patients were diagnosed with bacteriologically confirmed pulmonary TB, of whom 430 completed the directly observed treatment, short-course (DOTS) program at the study sites (Figure 1). Adherence to anti-TB therapy was supervised by the healthcare staff, in cooperation with the patients' family members, under the DOTS strategy of the national TB control program.

Of the 430 patients, 183 (42.6%) were aged <35 years, and 341 (79.3%) were male. HIV coinfection was seen in 22 patients (5.1%), 290 patients (70.2%) had cavities on chest radiographs at diagnosis, and 70 patients (16.9%) had infiltrates spreading to more than half of the lung zones. Among the 413 patients in whom MTB strains were genotyped, Beijing genotype strains were identified in 240 (58.1%) patients, of which 152 belonged to the ancient Beijing sublineages, accounting for 36.8% of all strains tested, and 88 strains belonged to the modern Beijing sublineages, accounting for 21.3% of all the strains tested (Table 1).

Treatment outcomes are illustrated in Figure 1. Treatment failure was noted in 17 (4.0%, 95% CI, 2.3–6.3) of the 430 patients. The remaining 413 patients were considered cured, and 403 of them entered the follow-up period. The median of follow-up time was 484 days (95% CI, 483–487 days) after completion of treatment. Recurrence was observed in 30 patients (7.4%, 95% CI, 5.1–10.5) during this period. Of these, 21 patients (70.0%) exhibited culture-positive results. Culture results were not available or were difficult to assess for the remaining 9 patients (30.0%), but all had smear-positive results with clinical/radiographic changes. All 30 patients with recurrent TB received a retreatment regimen of $2SHRZE/1HRZE/5H_3R_3E_3$. The median time to recurrence was 137 days (95% CI, 110–218 days) after the end of their previous treatment episode.

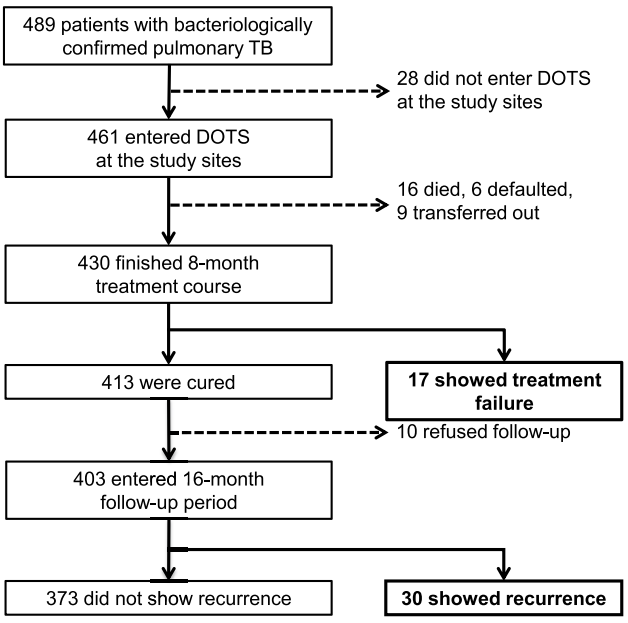

Figure 1. Study flow. TB: tuberculosis; MTB: *Mycobacterium tuberculosis*; NTM: non-tuberculous mycobacterium; DOTS: directly observed treatment, short-course.

Table 1
Characteristics of patients with smear-positive culture-positive pulmonary tuberculosis who finished an 8-month treatment course.

	Total number tested	Number of patients	%
Age (in years)	430		
<25		61	14.2
25–34.9		122	28.4
35–44.9		78	18.1
45–54.9		100	23.3
≥55		69	16.1
Sex	430		
Male		341	79.3
Female		89	20.7
Body mass index	430		
<16		56	13.0
16–18.4		176	40.9
≥18.5		198	46.1
Residential area	430		
Suburban		95	22.1
New urban		199	46.3
Old urban		136	31.6
Smoking habit	429		
Smoker		164	38.2
Ex-smoker		120	28.0
Nonsmoker		145	33.8
HIV status	428		
Positive		22	5.1
Negative		406	94.9
Infiltrate on chest radiograph	414		
≤3 zones		344	83.1
>3 zones		70	16.9
Cavity on chest radiograph*	413		
Yes		290	70.2
No		123	29.8
Drug resistant profile	430		
Sensitive to all four drugs tested†		272	63.3
Any resistance to isoniazid		113	26.3
Any resistance to streptomycin		111	25.8
Any resistance to rifampicin		15	3.5
Any resistance to ethambutol		9	2.1
Multidrug resistance		13	3.0
MTB lineage/sublineage	413		
Modern Beijing		88	21.3
Ancient Beijing		152	36.8
Non-Beijing		173	41.9

HIV: Human immunodeficiency virus; MTB: *Mycobacterium tuberculosis*.
* The number of patients from whom available information was obtained was listed. The information on cavitary lesion was available in 413 patients, although 414 chest x-ray films were taken.
† Includes isoniazid, streptomycin, rifampicin, and ethambutol.

3.2. Sublineages of Beijing genotype strains and treatment failure

MTB genotypes were divided into three categories: modern Beijing, ancient Beijing, and non-Beijing, including East African–Indian type. Univariate analysis indicated that infection with modern Beijing MTB strains was significantly associated with treatment failure [OR = 4.15 (95% CI, 1.01–17.00)], whereas infection with ancient Beijing strains was not, when non-Beijing types were set as a reference category. Treatment failure was significantly associated with resistance to SM, RMP, INH, or MDR; positivity of smear testing after the first 2 months of treatment; and the presence of infiltrates in more than half of the lung on chest radiographs. Age, sex, presence of cavities on radiographs, HIV status, and clustering status of the MTB strains were not significantly associated with treatment failure (Table 2).

Multivariate analysis indicated that RMP resistance was significantly associated with treatment failure [aOR = 6.64 (95% CI, 1.48–29.73)]; however, SM resistance, INH resistance, and infection with the modern Beijing sublineage were not (Table 2). When we replaced RMP and INH resistance by MDR in the same model, MDR

Table 2
Results of univariate and multivariate analyses using logistic regression models on sublineages of Beijing genotype and other factors evaluated for an association with treatment failure (n = 430).

	Proportion with treatment failure (%)	Univariate		Multivariate*	
		OR	95% CI	OR	95% CI
Age (increased by one year)		1.00	0.96–1.03		
Gender					
Male	15/341 (4.4)	1.00	(Reference)		
Female	2/89 (2.3)	0.50	0.11–2.23		
MTB strain					
Other strains	3/173 (1.73)	1.00	(Reference)	1.00	(Reference)
Ancient Beijing	6/152 (3.95)	2.33	0.57–9.48	1.38	0.31–6.11
Modern Beijing	6/88 (6.82)	**4.15**	**1.01–17.00**	3.71	0.86–15.96
Resistant to SM					
No	7/319 (2.2)	1.00	(Reference)	1.00	(Reference)
Yes	10/111 (9.0)	**4.41**	**1.64–11.90**	1.77	0.46–6.76
Resistant to RMP					
No	13/415 (3.1)	1.00	(Reference)	1.00	(Reference)
Yes	4/15 (26.7)	**11.24**	**3.16–40.07**	**6.64**	**1.48–29.73**
Resistant to INH					
No	7/317 (2.2)	1.00	(Reference)	1.00	(Reference)
Yes	10/113 (8.9)	**4.30**	**1.60–11.59**	2.34	0.62–8.90
Multidrug resistance					
No	13/417 (3.1)	1.00	(Reference)		
Yes	4/13 (930.8)	**13.81**	**3.76–50.72**		
Presence of infiltrate on CXR					
≤3 zones†	10/344 (2.9)	1.00	(Reference)		
>3 zones	6/70 (8.6)	**3.13**	**1.10–8.92**		
Presence of cavity on CXR					
No	1/123 (0.8)	1.00	(Reference)		
Yes	15/290 (5.2)	6.65	0.87–50.94		
Smear at month 2‡					
Negative	9/373 (2.4)	1.00	(Reference)		
Positive	8/56 (14.3)	**6.74**	**2.48–18.30**		
HIV infection					
No	16/406 (3.9)	1.00	(Reference)		
Yes	1/22 (4.6)	1.16	0.15–9.17		
Clustered MTB strains					
No	10/271 (3.7)	1.00	(Reference)		
Yes	5/142 (3.5)	0.95	0.32–2.84		

MTB: *Mycobacterium tuberculosis*; OR: odds ratio; 95% CI: 95% confidence interval; SM: streptomycin; RMP: rifampicin; INH: isoniazid; HIV: human immunodeficiency virus; CXR: chest X-ray.
Boldfaced values indicate odds ratios and 95% CI with statistical significance ($P < 0.05$).
* In the multivariate analysis, resistance to SM, RMP, and INH, MTB strains (ancient Beijing, modern Beijing and others) were included in the logistic models.
† Zones of the lung field.
‡ Two months after starting treatment.

was also significantly associated with treatment failure [aOR = 10.23 (95% CI, 2.27–46.15)] (table not shown). Smear test results at month 2 and chest X-ray (CXR) findings may represent disease severity [30] as a result of host–pathogen interaction or phenotypic consequences of variations in MTB lineages/sublineages [31]. Therefore, we did not include them in the final model for the purposes of the present study.

3.3. Sublineages of Beijing genotype strains and TB recurrence

First, patients infected with a Beijing strain had a significantly shorter time to TB recurrence, confirmed by the log-rank test ($P = 0.0211$) (Figure 2A). Second, patients with the modern Beijing strains had a significantly shorter time to TB recurrence than patients with non-Beijing strains ($P = 0.0213$) (Figure 2B). However, no other pairwise comparisons of sublineages were statistically significant (data not shown). The numbers of patients showing recurrence during the observation period were 9 (11.3%) among the 80 patients with the modern Beijing MTB strain, 13 (9.1%) among the 143 patients with the ancient Beijing strain, and 6 (3.6%) among the 165 patients with other (non-Beijing) strains. The proportions of patients with TB recurrence within one year (12 months) after completing treatment for the prior TB episode were 11.2% (95% CI, 6.0–20.3) in those infected with the modern Beijing MTB strain, 8.5% (4.9–14.4) in those with the ancient Beijing strain, and 3.7% (1.7–8.0) with other strains.

Assuming that coexisting drug resistance might influence the effect of Beijing MTB lineages/sublineages on recurrence, we next investigated the effects of recurrence-associated factors using Cox proportional hazard models for univariate and multivariate analyses. Using the non-Beijing strains as the reference group, infection with the modern Beijing strains was significantly associated with time to TB recurrence in the univariate analysis [HR = 3.16 (95% CI, 1.13–8.89)] and the multivariate analysis with adjustment for drug resistance [aHR = 3.29 (95% CI, 1.17–9.27)]. Infection with the ancient Beijing strains was not significantly associated with time to TB recurrence (Table 3).

Resistance to RMP or INH was not significantly associated with time to TB recurrence. Resistance to SM was significantly associated with time to TB recurrence in the univariate model [HR = 2.50 (95% CI, 1.21–5.15)], but not in the multivariate model (Table 3). When we replaced RMP and INH resistance by MDR in the same model, MDR was also not significantly associated with recurrence [aHR = 3.25 (95% CI, 0.71–14.94)] (table not shown). Other characteristics, including HIV coinfection, severity on CXR, and category of patients' residential areas, were not associated with time to TB recurrence (Table 3).

4. Discussion

Our study demonstrated that RMP resistance was associated with treatment failure among patients newly diagnosed with smear-positive and culture-positive pulmonary TB. In addition, we found that infection with a modern Beijing strain was a risk factor for TB recurrence within 16 months of follow-up after completing treatment for a previous TB episode. In our study population, the prevalence of INH and SM resistance was higher among patients infected with ancient Beijing strains than among those infected with modern Beijing strains; however, INH resistance and SM resistance or infection with ancient Beijing strains were not associated with the increased risk of treatment failure or TB recurrence.

Four percent of TB patients had a treatment failure in our study, similar to 4.3% of TB patients who failed treatment in a different study conducted in southern Vietnam [32], and 5% in a multicenter trial conducted in eight centers in Africa and Asia [33]. These treatment failure rates are higher than those (2%) reported globally [1], in part because of the relatively less effective treatment regimen, 2S(E)HRZ/6HE, that was used during the study period at these study sites. The proportion of TB recurrence was 7.4% in the present study, versus 8.6% in another study conducted in northern Vietnam [34], 6.5% in southern Vietnam [32], and 5% in the multicenter trial [33] cited above. The longer follow-up period (32 months as maximum) and less strict criteria used for diagnosis of TB recurrence in the northern Vietnamese study [34] may explain the higher proportion of recurrence in that study compared with our study. Conversely, active monitoring using periodical sputum testing during the follow-up period may have improved our detection of TB recurrence, compared with the passive case finding of TB recurrence used in the study in southern Vietnam [32].

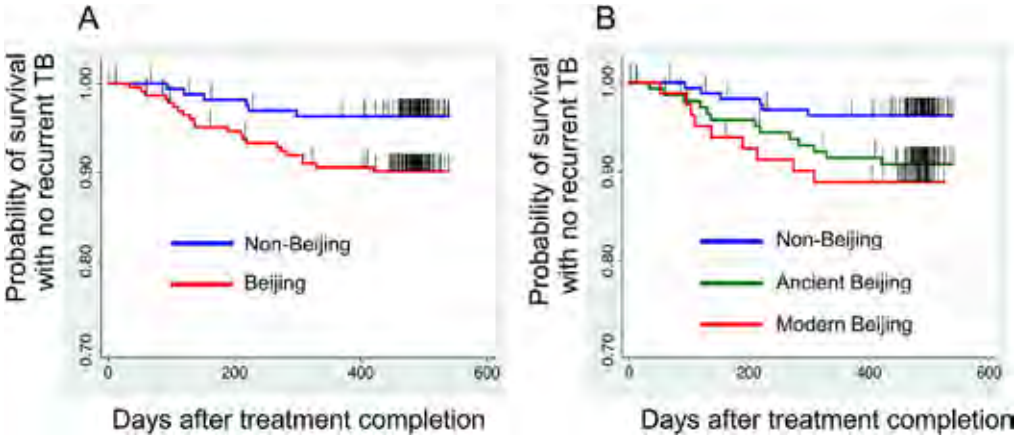

Figure 2. (A) Kaplan–Meier curves illustrating Beijing and non-Beijing MTB lineages and time to recurrence as indicated by the probability of survival with no recurrent tuberculosis, (B) Kaplan–Meier curves illustrating modern Beijing, ancient Beijing sublineages, non-Beijing lineage, and time to recurrence as indicated by the probability of survival with no recurrent tuberculosis. Time to recurrence between the Beijing and non-Beijing groups ($P = 0.0211$ by log rank test). Time to recurrence between the modern Beijing and non-Beijing groups ($P = 0.0213$ by log rank test) but not significant in other combinations, including modern Beijing and ancient Beijing sublineages (data not shown). TB: tuberculosis.

Table 3
Results of univariate and multivariate analyses using proportional hazard models on sublineages of Beijing genotype and other factors evaluated for an association with TB recurrence (n = 403).

	Proportion with recurrence (%)	Univariate		Multivariate*	
		HR	95% CI	HR	95% CI
Age (increased by one year)		1.00	0.98–1.03		
Gender					
Male	23/316 (7.3)	1.00	(Reference)		
Female	7/87 (8.1)	1.15	0.50–2.69		
MTB strain					
Other strains	6/165 (3.64)	1.00	(Reference)	1.00	(Reference)
Ancient Beijing	13/143 (9.09)	2.55	0.97–6.70	2.08	0.77–5.62
Modern Beijing	9/80 (11.25)	**3.16**	**1.13–8.89**	**3.29**	**1.17–9.27**
Resistant to SM					
No	17/306 (5.6)	1.00	(Reference)	1.00	(Reference)
Yes	13/97 (13.4)	**2.50**	**1.21–5.15**	2.33	0.97–5.61
Resistant to RMP					
No	28/394 (7.1)	1.00	(Reference)	1.00	(Reference)
Yes	2/9 (22.2)	3.63	0.86–15.26	3.1	0.66–14.55
Resistant to INH					
No	20/304 (6.6)	1.00	(Reference)	1.00	(Reference)
Yes	10/99 (10.1)	1.58	0.74–3.38	0.85	0.33–2.19
Smear at month 2†					
Negative	24/355 (6.8)	1.00	(Reference)		
Positive	6/48 (12.5)	1.84	0.75–4.50		
Clustered					
No	15/255 (5.9)	1.00	(Reference)		
Yes	13/133 (9.8)	1.64	0.78–3.45		
Presence of infiltrate on CXR					
≤3 zones‡	26/327 (8.0)	1.00	(Reference)		
>3 zones	4/62 (6.5)	0.79	0.27–2.25		
Presence of cavity on CXR					
No	11/119 (9.2)	1.00	(Reference)		
Yes	19/269 (7.1)	0.75	0.36–1.59		
HIV infection					
No	29/380 (7.6)	1.00	(Reference)		
Yes	1/21 (4.8)	0.66	0.09–4.85		

TB: tuberculosis; MTB: *Mycobacterium tuberculosis*; HR: hazard ratio; 95% CI: 95% confidence interval; SM: streptomycin; RMP: rifampicin; INH: isoniazid; HIV: human immunodeficiency virus; CXR: chest X-ray.
Boldfaced values indicate hazard ratios and 95% CI with statistical significance ($P < 0.05$).
* In the multivariate analysis, resistance to SM, RMP, and INH, MTB strains (ancient Beijing, modern Beijing and others) were included in the Cox models.
† Two months after starting treatment.
‡ Zones of the lung field.

In the present study, RMP resistance was strongly associated with treatment failure but not with recurrence. The majority of RMP-resistant strains had MDR (22/24 or 91.7%), which is a well-known risk factor for treatment failure [35]. In some resource-limited settings, drug susceptibility testing is not performed routinely for new patients before starting treatment. In those settings, application of necessary countermeasures against MDR, including a timely change of the treatment regimen, should be considered once treatment failure is suspected.

Also in the present study, active disease caused by the Beijing genotype was associated with an increased risk of TB recurrence, which was consistent with previous reports from different areas of the world [10–13]. Interestingly, modern Beijing strains exhibited a potential risk for recurrence when non-Beijing MTB genotypes were set as the reference group. Other investigators [36] have previously reported the high prevalence of modern Beijing strains among patients with a history of anti-TB treatment, suggesting that this sublineage may be associated with TB recurrence. Our study results suggest that the modern Beijing strains influenced TB recurrence, whereas the ancient Beijing strains did not. There was no significant difference in the probability of recurrent TB in patients with ancient versus modern Beijing sublineages. This result could be due to the low number of recurrent TB patients with the ancient sublineage in our study, as well as insufficient statistical power.

Virulent phenotypes of the modern Beijing sublineage strains could be differentiated by frequent drug resistance mutations and propagation, or disease development with a short latency period based on the host–pathogen interaction. In our study, the modern Beijing sublineage was actually an independent risk factor regardless of drug resistance status in the multivariate analysis. Disease caused by modern Beijing MTB strains conferred a risk of TB recurrence, despite having less frequent drug resistance than that caused by the ancient Beijing genotype. Previous reports indicate that modern Beijing strains produce lower levels of proinflammatory cytokines [37,38] and have a higher survival in macrophages [38] than ancient Beijing strains. These factors may facilitate persistent infection or reactivation from latent infection, although investigation of these properties at sublineage levels has only just begun. Nevertheless, these findings could help explain

their potential to dysregulate host defense mechanisms, leading to increased virulence and successful spread of the modern sublineage [14,15,17].

SM resistance unexpectedly exhibited a significant association with recurrence in the univariate analysis but not in the multivariate analysis. In another study conducted in southern Vietnam [13], resistance to INH was a risk factor for recurrence. Although direct evidence is lacking, this inconsistency may be due to area-dependent differences in the distribution patterns of major Beijing sublineages and their subgroups. Resistance to SM cannot be overlooked because this antibiotic is still used as a component of treatment regimens, such as 2SHRZE/1HRZE/5HRE, recommended by WHO for severe TB or retreated TB cases [39], in which relapse is often observed.

Other factors (e.g., poor treatment adherence, increased disease severity by expansion of lesions in the lung, HIV coinfection [4], diabetic status [40], and clustering status possibly indicating recent transmission) were not associated with TB recurrence by univariate analysis in our study ($P > 0.2$, data not shown). For this reason, they were not included in the multivariate analysis. Positive smear results at month 2 were significantly associated with treatment failure. This was consistent with the classical idea that persistently positive smear testing after the intensive treatment phase is a possible surrogate for treatment outcome [41].

The present study has some limitations. First, we were not able to differentiate relapse from reinfection among recurrent cases. However, we presume they were true relapse cases because most of the second TB episodes appeared in a relatively short time after cure; the median of time to recurrence was 137 days (95% CI, 110–218 days) after the end of treatment. Second, we were unable to study the host immune response to clarify the mechanism(s) underlying the association between modern Beijing sublineage and recurrence. Nevertheless, this is the first report from Vietnam on the association between MTB sublineages and unfavorable treatment outcomes, including recurrence. Future subclassification of Beijing sublineages through the analysis of genome-wide variations may help clarify the genotype–phenotype relationship and may provide an explanation for the inconsistent results previously reported. In addition, the strength of our cohort study was supported by a high proportion of patients completing treatment, followed up actively and intensively, and with clinicoepidemiological data linked to MTB lineages/sublineages with drug-resistance profiles.

In conclusion, among patients newly diagnosed with smear-positive pulmonary TB, infection with the modern Beijing sublineage was associated with recurrence, whereas infection with the ancient Beijing sublineage was not. Considering the high prevalence of circulating Beijing genotype strains and pretreatment drug resistance in the study area, multiple factors should be considered to achieve better TB management.

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Dynamics of immune parameters during the treatment of active tuberculosis showing negative interferon gamma response at the time of diagnosis

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SUMMARY

Objectives: In the performance of interferon gamma release assays (IGRA) for the diagnosis of tuberculosis (TB) infection, false-negative results are a major obstacle. In active TB patients, treatment-dependent changes of the negative test results remain unknown.
Methods: The treatment course of 19 smear-positive/culture-confirmed TB patients who had IGRA-negative results by QuantiFERON-TB in-tube (QFT-IT) method at the time of diagnosis (month 0) in a previous study, were monitored in the present study. Blood was further collected at months 2 and 7, and the concentrations of 27 immune molecules were measured in the plasma supernatants remaining after performing the IGRA, using a suspension array system.
Results: After initiating treatment, eight of the 19 QFT-IT-negative patients showed positive conversion, whereas the remaining 11 (58%) did not; the interferon gamma (IFN-γ) response was restored to levels higher than 1 IU/ml in only three of the eight patients with positive conversion. Plasma concentrations of interleukin 1 receptor antagonist, interleukin 2, and interferon gamma-induced protein 10 remained low after *Mycobacterium tuberculosis*-specific antigen stimulation at months 2 and 7 in the continuously QFT-IT-negative group, whereas the parameters were elevated only in the transiently QFT-IT-negative group.
Conclusions: It was demonstrated that a majority of active TB patients showing negative IGRA results did not regain sufficient levels of immune responsiveness despite successful treatment.

1. Introduction

The interferon gamma release assay (IGRA) is currently used as one of the representative tests to diagnose tuberculosis (TB) infection.[1] In this test, the cellular response to *Mycobacterium tuberculosis* is assessed by measuring the interferon gamma (IFN-γ) released from peripheral blood lymphocytes after stimulation with *M. tuberculosis*-specific antigens.[1]

The QuantiFERON-TB Gold In-Tube test (QFT-IT) is a commercially available IGRA based on the ELISA method; it has a sensitivity of 78–83% and specificity of 98–100%.[1] This imperfect sensitivity causes difficulties in ruling out TB infection, particularly when the

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prevalence of TB infection is high, and the low negative predictive value of the test may reduce the chance of a possible therapeutic intervention.

To assess the sensitivity of the QFT-IT, patients with bacteriologically proven active TB disease have often been recruited as surrogates for individuals with latent TB infection. The IGRA results also provide a clue to suspect active TB disease clinically.

Although weakened immunity in severe TB may affect the test results,[2] treatment-dependent changes of the negative IGRA results with a very low IFN-γ response have not been investigated fully.

This study group has recently reported the results of a cross-sectional study on the sensitivity of the QFT-IT method in Hanoi, Vietnam, and demonstrated that aging, emaciation, HIV co-infection, and a particular HLA genotype, DRB1*07:01, lowered the sensitivity of the test in active pulmonary TB patients.[3] In the present study, 19 of the 24 patients who showed false-negative results at the time of diagnosis were monitored. Further analysis of the treatment response and dynamics of immune parameters was performed, with the measurement of the concentrations of various cytokines and chemokines in the plasma supernatants remaining after use in the IGRA assay.

2. Methods

2.1. Study subjects and IGRA

From July 2007 to March 2009, whole blood was collected from 504 adult patients in Hanoi, Vietnam, who had smear-positive/culture-confirmed pulmonary TB and a history negative for TB treatment. The blood was collected in heparinized tubes before anti-TB treatment was initiated (month 0).[3] The patients were tested with a commercially available ELISA-based IGRA (QFT-IT; Cellestis, Victoria, Australia), as reported previously.[3] Plasma supernatants were separated at 4000 rpm for 15 min (Model 2010; Kubota Co., Tokyo, Japan) and stored at −80 °C until measurement. The cut-off value to interpret the QFT-IT results was set at 0.35 IU/ml, as per the manufacturer's instructions.

In the present study, further blood samples for QFT-IT and other tests were collected and served for analysis at two more time points: after the initial phase of treatment (month 2) and close to the end of treatment (month 7). Positive conversion of the IGRA was defined by a negative result at month 0 and positive result(s) at month 2, month 7, or both time points.

2.2. Clinical data collection

The extents of cavitary lesions and infiltrates were also semi-quantitated by the grading method.[4] *M. tuberculosis* isolates were analyzed by single nucleotide polymorphism (SNP) and spoligotyping methods.[5]

2.3. Treatment course

Following the national standard regimen at that time, all patients received an 8-month course of the anti-TB treatment regimen 2S(E)HRZ/6HE, which was commonly administered during the study period in Vietnam.[5]

2.4. Immune analyte profiling by Bio-Plex assay and adiponectin ELISA assay

Immune molecules released into the plasma after TB antigen stimulation were estimated from their concentrations after a 16- to 24-h incubation with TB-specific antigens (TBAg) minus those with no antigens (Nil) obtained from the QFT-IT method. Their concentrations were determined using a human 27-plex assay (14 cytokines: interleukin (IL)-1β, IL-1 receptor antagonist (IL-1RA), IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IFN-γ, tumor necrosis factor alpha (TNF-α); seven chemokines: eotaxin, IL-8, IFN-γ-inducible protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory proteins MIP-1α and MIP-1β, RANTES; and six growth factors: IL-7, fibroblast growth factor (FGF) basic, granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony stimulating factor (GM-CSF), platelet-derived growth factor (PDGF)-BB, vascular endothelial growth factor VEGF) (Bio-Plex Suspension Array System; Bio-Rad, Hercules, CA, USA), following the manufacturer's instructions. All samples, standards, and controls were run in duplicate and manipulated in accordance with the manufacturer's protocol. Samples were diluted in a 1:4 volume ratio with the sample diluent and incubated for 30 min at room temperature; they were then agitated at 300 rpm to be captured with antibody-coupled magnetic beads. Following three washes in a Bio-Plex Pro Wash Station, the samples were incubated with biotinylated detection antibodies and agitated at 300 rpm in the dark for 30 min at room temperature. Each captured analyte was detected by the addition of streptavidin–phycoerythrin and quantified using a Bio-Plex array reader. The fluorescence intensities in the samples and known standards were acquired and converted to the plasma concentrations of each analyte using the Bio-Plex 200 System software (version 6.0; Bio-Rad Laboratories). When the induction of immune molecules after TB antigen stimulation was below their detection limits, these molecules were excluded from subsequent analysis.

Total human adiponectin (low, middle, and high molecular weight) levels in plasma were also measured using the Quantikine Human Total Adiponectin/Acrp30 Immunoassay Kit (R&D Systems, Inc., Minneapolis, MN, USA). The mean minimum detectable dose was 0.246 ng/ml.

2.5. Statistical analysis

Values including cytokine concentrations among groups were analyzed by Kruskal–Wallis tests with multiple comparisons for all pairs by Steel–Dwass method. The inequality of proportions among the groups was analyzed by Fisher's exact test. The statistical analysis was performed using Stata version 12 (Stata Corp, College Station, TX, USA) and JMP 9 (SAS Institute Inc., Cary, NC, USA). A p-value of < 0.05 was considered to be statistically significant. The Bonferroni correction was also used for multiple comparisons, when appropriate.

3. Results

3.1. Characteristics of the patients who completed the three-time blood collection stratified by IGRA-negative or positive result at month 0

After the cross-sectional study reported previously,[3] 19 of the 24 IGRA-negative patients with culture-confirmed active pulmonary TB at the time of diagnosis completed the three-time blood collection at months 0, 2, and 7; these patients were thus analyzed in the present study. The 351 patients who initially showed QFT-IT-positive results and completed the three-time blood collection were set as a reference. Samples showing indeterminate results before treatment were omitted from this analysis.

As expected from the results of the previous report,[3] increasing age, low body mass index (BMI) at the time of diagnosis, and the HLA-DRB1*07:01 allele were observed more frequently in the 19 QFT-IT-negative patients than in the 351 QFT-IT-positive

patients before treatment (**Supplementary Material**, Online Resource 1). HIV co-infection was nearly significantly associated with QFT-IT-negative results ($p = 0.0541$). In addition, it was possible to combine QFT-IT results with *M. tuberculosis* strain genotypes in 90% of these patients. Approximately 60% of the *M. tuberculosis* strains were of the Beijing genotype in this population, according to the previous results of SNP analysis and spoligotyping.[5] Non-Beijing strains, particularly EAI strains, were predominant in the QFT-IT-negative group as compared to the QFT-IT-positive group (52.9% vs. 18.3%; $p = 0.0004$; **Supplementary Material**, Online Resource 1).

3.2. Treatment course of the patients stratified by IGRA-negative or positive result at month 0

During treatment, QFT-IT IFN-γ values (TBAg minus Nil) of the 19 patients who showed QFT-IT-negative results at month 0 remained relatively low (Table 1).

The number of patients with a positive sputum smear was relatively small at month 7, indicating that a majority of the patients in both groups had been treated successfully; only one of 19 (5.3%) and five of 351 (1.4%) patients were regarded as treatment failure in the QFT-IT-negative and positive groups, respectively, at month 0, and this difference was not significant (data not shown). The extent of cavitary lesions and infiltrates on chest X-ray at month 0 and month 7 were also not different between the QFT-IT-negative and positive groups (Table 1).

3.3. Characteristics of the IGRA-negative patients at month 0 stratified by the absence or presence of positive conversion during treatment

Eight of the 19 QFT-IT-negative patients showed positive conversion at month 2 or month 7, whereas the remaining 11 (58%) did not. Background information, such as age and low BMI, was further compared between these two subgroups, but no particular characteristics were statistically significant (Table 2). Only three of the eight patients with positive conversion showed IFN-γ values higher than 1 IU/ml at month 2 (1.0, 2.2, and 3.2 IU/ml) and month 7 (1.8, 5.2, and 10.0 IU/ml). These patients did not carry any of the DRB1*07:01 alleles (data not shown), whereas all of the patients carrying the HLA-DRB1*07:01 allele in the QFT-IT-negative group showed IFN-γ values lower than 1.0 IU/ml throughout the treatment period (data not shown).

During treatment, sputum smear and chest X-ray findings were not significantly different between the subgroups with and without positive conversion. A trace of cavitary lesions was not observed in any of the five patients without positive conversion, but was observed in three of the six patients with positive conversion (**Supplementary Material**, Online Resource 2), although this difference was not statistically significant ($p = 0.18$).

3.4. Treatment-dependent changes in immunological parameters in the blood from patients with IGRA-negative results before treatment

The concentrations of immune molecules induced by *M. tuberculosis* antigen-specific antigens were analyzed in patients

Table 1
Treatment response of the patients stratified by QFT-IT-negative or positive result at month 0

		Negative result before treatment ($n = 19$)		Positive result before treatment ($n = 351$)		p-Value
QFT-IT-positive result	Month 0	0	(0.0%)	351	(100.0%)	<0.0001
	Month 2	5	(26.3%)	312	(88.9%)	<0.0001
	Month 7	6	(31.6%)	300	(85.5%)	<0.0001
QFT-IT value (IU/ml)	Month 0	0.21	(0.05–0.25)	8.61	(3.46–14.9)	<0.0001
	Month 2	0.11	(0.03–0.52)	3.80	(1.15–11.64)	<0.0001
	Month 7	0.19	(0.02–0.79)	2.85	(0.88–8.82)	<0.0001
Positive sputum smear	Month 0	19	(100.0%)	351	(100.0%)	1.0000
	Month 2	4	(21.1%)	40	(11.4%)	0.2623
	Month 5	1	(5.3%)	6	(1.7%)	0.3107
	Month 7	1	(5.3%)	5	(1.4%)	0.3107
Chest X-ray						
Extent of cavity (number of affected zones of the lung field)[a]	Month 0					0.6753
	0	5	(27.8%)	105	(31.2%)	
	1	9	(50.0%)	174	(51.6%)	
	2	3	(16.7%)	46	(13.7%)	
	3	1	(5.6%)	8	(2.4%)	
	>4	0	(0.0%)	4	(1.2%)	
	Month 7					0.1304
	0	8	(72.7%)	234	(91.8%)	
	1	3	(27.3%)	19	(7.5%)	
	2	0	(0.0%)	1	(0.4%)	
	3	0	(0.0%)	1	(0.4%)	
	>4	0	(0.0%)	0	(0.0%)	
Extent of infiltration (number of affected zones of the lung field)[b]	Month 0					0.1723
	0	2	(11.1%)	16	(4.7%)	
	1	3	(16.7%)	81	(23.9%)	
	2	4	(22.2%)	117	(34.5%)	
	3	3	(16.7%)	73	(21.5%)	
	>4	6	(33.3%)	52	(15.3%)	
	Month 7					0.3157
	0	3	(27.3%)	106	(41.7%)	
	1	4	(36.4%)	99	(39.0%)	
	2	4	(36.4%)	33	(13.0%)	
	3	0	(0.0%)	11	(4.3%)	
	>4	0	(0.0%)	5	(2.0%)	

QFT-IT, QuantiFERON-TB in-tube.
[a] $n = 18$ and $n = 337$ at month 0; $n = 11$ and $n = 255$ at month 7.
[b] $n = 18$ and $n = 339$ at month 0; $n = 11$ and $n = 254$ at month 7.

Table 2
Characteristics of QFT-IT-negative patients at month 0 stratified by the absence or presence of positive conversion

		With no positive conversion during treatment ($n=11$)		With positive conversion during treatment ($n=8$)		p-Value
Sex	Male	11	(100.0%)	7	(87.5%)	0.4211
	Female	0	(0.0%)	1	(12.5%)	
Age at diagnosis, years		55.4	(37.4–67.0)	43.3	(30.8–49.0)	0.1167
Body mass index, kg/m^2		16.7	(15.8–19.1)	15.9	(13.1–17.8)	0.3637
Smoking habit	Smoker/ex-smoker	8	(72.7%)	6	(75.0%)	1.0000
	Non-smoker	3	(27.3%)	2	(25.0%)	
BCG vaccination[a]	No	1	(9.1%)	1	(12.5%)	1.0000
	Yes	1	(9.1%)	0	(0.0%)	
	Unknown	9	(81.8%)	7	(87.5%)	
Sputum smear	Scanty	2	(18.2%)	0	(0.0%)	0.7611
	+	4	(36.3%)	4	(50.0%)	
	++	3	(27.3%)	3	(37.5%)	
	+++	2	(18.2%)	1	(12.5%)	
HIV status	Negative	9	(81.8%)	7	(87.5%)	1.0000
	Positive	2	(18.2%)	1	(12.5%)	
White blood cell count $\times 10^9$/l		8.6	(5.3–12.0)	9.9	(9.5–18.8)	0.2151
Lymphocyte count $\times 10^9$/l		1.4	(0.8–2.0)	2.1	(1.7–2.5)	0.1345
Drug resistance	Isoniazid	1	(9.1%)	1	(12.5%)	1.0000
	Rifampicin	1	(9.1%)	0	(0.0%)	1.0000
	Streptomycin	2	(18.2%)	1	(12.5%)	1.0000
	Ethambutol	1	(9.1%)	0	(0.0%)	1.0000
HLA-DRB1*07:01 allele number	0	6	(54.5%)	6	(75.0%)	0.1852
	1	4	(36.4%)	0	(0.0%)	
	2	1	(9.1%)	2	(25.0%)	
MTB strains[b]	Beijing	1	(11.7%)	2	(25.0%)	0.5335
	EAI	6	(66.7%)	3	(37.5%)	
	Other	2	(22.2%)	3	(37.5%)	

QFT-IT, QuantiFERON-TB in-tube; BCG, bacille Calmette–Guérin; MTB, *Mycobacterium tuberculosis*.
[a] Self-declaration.
[b] $n=9$ and $n=8$.

showing negative results at month 0 with positive conversion at months 2 or 7, in patients showing negative results at month 0 without positive conversion, and in 21 randomly selected patients who showed a QFT-IT-positive result at month 0, and then completed the treatment course.

Of the 27 immune analytes in the plasma supernatants, IL-1RA, IL-2, IFN-γ, and IP-10 showed differences in concentrations among the groups, even after Bonferroni correction; these immunological parameters were predominantly induced in the QFT-IT-positive reference group, whereas their concentrations in QFT-IT-negative subgroups were low (Figure 1). Although the induction levels of these immune parameters gradually decreased during treatment in the QFT-IT-positive group, significant differences remained between the QFT-IT-positive group and the continuously negative group without positive conversion. In the transiently negative group with positive conversion, levels of these immune parameters were restored to some extent during the course of treatment; concentrations of IL-1RA and IL-2 in the transiently negative group were significantly higher than those in the continuously negative group at month 7 (Figure 1). Concentrations of IL-10, known as a regulatory cytokine, were not significantly different between these two subgroups; adiponectin concentrations did not show a significant difference either (data not shown).

4. Discussion

The QFT-IT test results of Vietnamese patients with culture-confirmed active pulmonary TB enrolled in a cross-sectional study were recently reported,[3] and factors that possibly lower the sensitivity of this ELISA-based IGRA were identified: aging, emaciation, HIV co-infection, and a particular HLA genotype, DRB1*07:01.[3] In the present study, the IGRA-negative TB patients from the previous study underwent continued monitoring during treatment, and treatment-dependent changes in immune status were characterized by measuring the concentrations of cytokines and chemokines in the plasma supernatants.

IL-1RA, IL-2, and IP-10, as well as IFN-γ, were more or less inducible after TB antigen-specific stimulation (TBAg minus Nil) in active pulmonary TB. The induction levels were significantly lower in the continuously IGRA-negative group than in the IGRA-positive group, even after initiating treatment. This is possibly due to immune regulatory mechanism(s), including impaired antigen presentation through a particular HLA molecule or long-lasting T-cell anergy,[6,7] although immunoregulatory IL-10 levels relevant to the regulatory T-cells were not different between these groups.

The difference in cytokine/chemokine induction was gradually lost between the IGRA-positive group and the transiently IGRA-negative group. The immune dynamics in the patients in whom the immune response was restored may be consistent with those of an early report, which showed the suppression of IFN-γ production from CD4+ T-cells in response to TB-specific peptides in patients with severe pulmonary TB before treatment.[2] However, the IFN-γ response was fully restored (>1 IU/ml) during the treatment period in only three of the 19 patients (16%).

It has thus been demonstrated that IGRA-negative patients tend to have continuously low immune responsiveness despite successful treatment, measuring not only IFN-γ but also other immune molecules. These findings indicate that factors unaffected by treatment are important when negative IGRA results are interpreted in active TB.

IL-1RA is a natural antagonist of IL-1 produced by activated monocyte/macrophages, and blocks the binding of IL-1α and IL-1β to the type I IL-1 receptor, without exerting agonist activity. IL-1RA is elevated in the blood of patients with inflammation, and IL-1RA has been suggested as a marker for TB disease activity and response to treatment.[8] Increased levels of IL-1RA were found both in the lung epithelial lining fluid and in the blood from patients with active pulmonary TB.[9] IL-1 is not easily detected in the circulation during human disease, and IL-1RA may be a better maker than IL-1 as an indicator of ongoing inflammation.[10]

The restoration of IL-2 production in the IGRA-negative group with positive conversion is consistent with previous reports:[11,12]

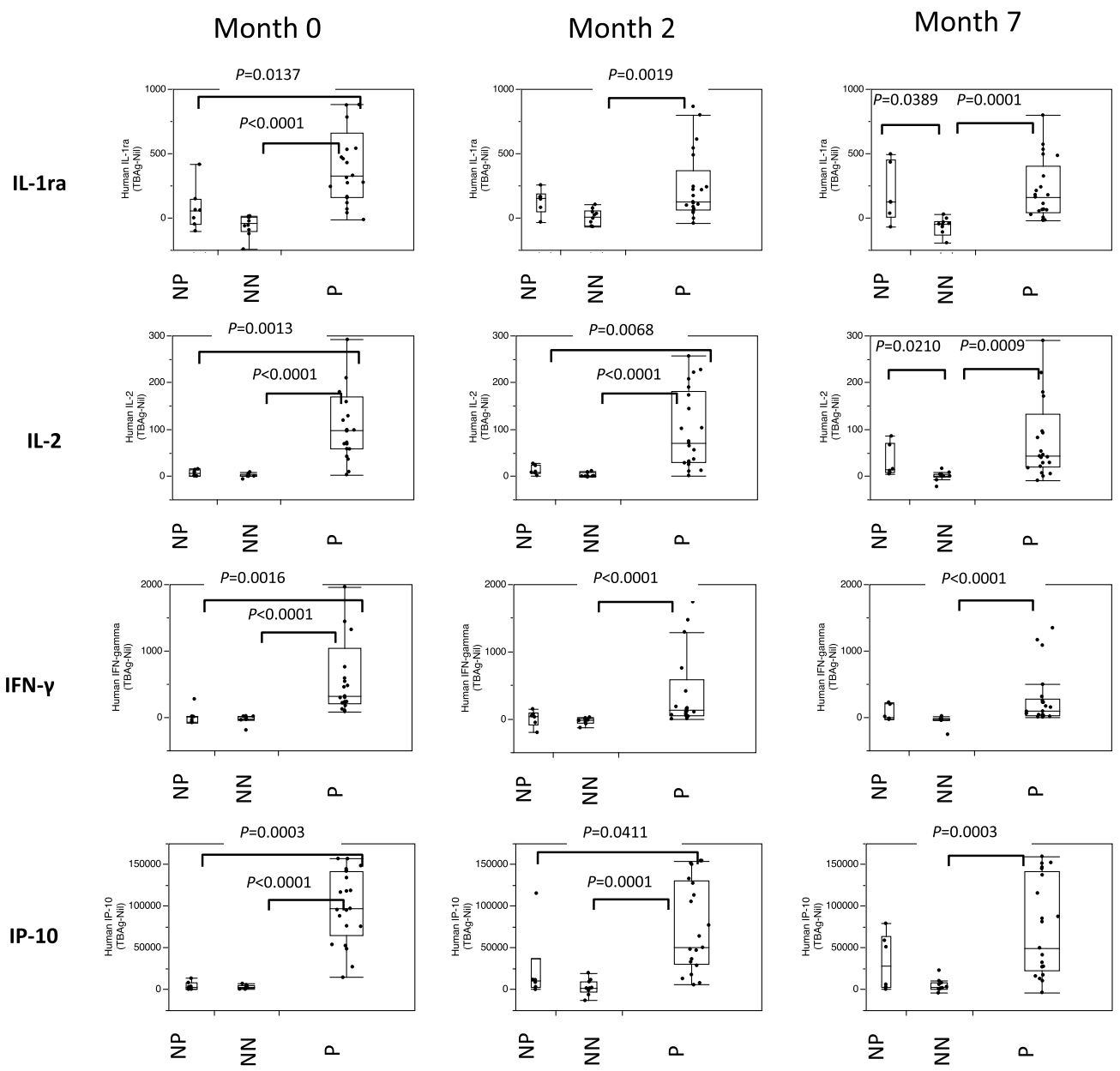

Figure 1. Immune analyte concentrations after TB antigen-specific stimulation at months 0, 2, and 7. Immune analyte concentrations (ng/ml) after TB antigen-specific stimulation (TBAg minus Nil) were compared among IGRA-negative groups at month 0 with positive conversion at months 2 or 7 (NP) and without positive conversion (NN), and the IGRA-positive reference group at month 0 (P). A significant difference in IL-1RA, IL-2, IFN-γ, and IL-2 concentrations was observed even after Bonferroni correction (uncorrected $p < 0.0005$ by Kruskal–Wallis test). All pairs were further compared by Steel–Dwass method, and their significant p-values are shown.

in patients with normal TB immunity, it is known that antigen-specific IFN-γ-only-secreting effector T-cells are predominant before treatment and dual IFN-γ/IL-2-secreting or polyfunctional T-cells with memory cell characteristics become predominant after starting treatment.[11,12] In the continuously IGRA-negative group, effector memory T-cells might have failed to expand for unknown reasons. Indeed, when the suspension array data were analyzed, low IL-2 induction in QFT-IT was observed together with the low IFN-γ response in the patients without positive conversion.

IP-10 is a chemokine expressed by antigen-presenting cells in response to IFN-γ, and IP-10-based tests are comparable with the IGRA response[13] before and during treatment.[14] This study group has previously reported that circulating adiponectin may be a marker for the severity of the disease,[15] but this parameter was not clearly associated with IGRA results in the present study.

Changes in clinical phenotypes relevant to treatment failure, such as chest radiographic lesions and smear-negative conversion, were not associated with the IGRA results at month 0. In the present study, the transiently IGRA-negative group with positive conversion did not show any clinical backgrounds distinct from those of the continuously IGRA-negative group without positive conversion, presumably because disease severity is further affected by the combination of other factors including patient age, patient BMI, and the bacterial burden.

Furthermore, *M. tuberculosis* strains of non-Beijing EAI genotype were frequently observed in the IGRA-negative group in this study. This may be partly confounded by the fact that non-Beijing strains are often observed in elderly people in Hanoi.[16] However, confirmation based on multivariable analysis was not performed because of the small sample size.

The HLA-DRB1*07:01 allele, an endogenous genetic factor, has previously been reported to be associated with QFT-IT false-negative results or a low IFN-γ response.[3] This tendency was shown throughout the treatment course in the present study, although it did not reach statistical significance, presumably due to the relatively small sample size.

These findings will provide insights into factors affecting QFT-IT false-negative results over a long term, although they may not be extrapolated to those of another IGRA, the T-SPOT.TB. Nevertheless, a variety of factors that potentially suppress the IGRA response should be investigated further and the test performance of the IGRA should be improved in future studies.

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Ethics statement: The study was approved by the ethics committees of the Ministry of Health, Vietnam, National Center for Global Health and Medicine, and The Research Institute of Tuberculosis, JATA Japan. All participants were enrolled upon provision of written informed consent.

Conflict of interest: The authors have declared that no competing interests exist.

Author contributions: IM carried out the immunoassays, drafting the paper. NTLH participated in supervising the on-site implementation of the study. LTH and DBT carried out the immunoassays. LTL and PHT participated in conception, design and supervision of the study. VCC participated in supervising the on-site implementation of the study. MH participated in analysis and interpretation of the data. NKo and SS participated in conception and design of the study. KH and NH were responsible for technical transfer and supervision. NK was responsible for the conception, design and overall supervision of the study, analysis and interpretation of data, drafting the paper or substantially revising it. All authors read and approved the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2015.09.021.

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Original article

Assessment of Health Systems in Relation to Interface Between Malaria Control Programs and Health System Strengthening: Comparative Study Between Nepal and Viet Nam

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Abstract

Introduction: Malaria control has been a major health issue with high priority in endemic countries and various efforts have been made with the support of foreign assistant partners. In order to implement efficient and sustainable control, integration of the control program into general health system or effective interactions between them is one of the important strategies.

Methods: Studies were conducted in Nepal and Viet Nam. Information obtained from document reviews, interviews, and field surveys were analyzed from the viewpoint of interface between malaria control program and the health system in accordance with six building blocks of a health system, with special emphasis on good practices and challenges in the implementation of the malaria control program.

Results: Among good practices, strong government commitment towards the control programs to strengthen facilities and capacity of health workers at the primary level, utilization of health volunteers, setting up mobile team and intensified education for residents were noteworthy. Key challenges mainly involved remote areas. Introduction of malaria due to population movement and the emergence of new endemic areas have become growing issues. While strengthening of the vertical health program appeared to have some impact on the general health system, particularly at the primary level, dissociation between the vertical control program and horizontal general health system still remains.

Conclusion: It is crucial to implement an effective and equitable malaria control program that responds to these existing challenges and can create a sustainable health system. Addressing these issues will lead to further strengthening of the health system there and eventually lead to the effective implementation of various health programs.

Key words: Malaria control, Health system, Nepal, Viet Nam

Introduction

Over the past decades malaria control has been implemented intensively with the support of Global Fund against AIDS, Tuberculosis and Malaria (GFATM)and other assistant partners as one of the most important disease control programs, and good results have been obtained in some endemic countries.[1-3] Malaria control programs have been implemented under global malaria control strategies although key strategies have varied over times: spraying

of pesticide, community-based control, school health-based control, Roll Back Malaria etc. In recent years, the importance of disease control measures in relation to health system strengthening (HSS), particularly the integration of a control program (vertical health system) into the general health system (horizontal health system) with the aim of achieving a synergic effect among health programs, has been stressed as an important strategy.[4-6]

In the process of disease control, good practices have been recognized, but interaction between disease specific control programs and HSS has been debated, namely whether disease specific programs have actually contributed to the strengthening of the general health system and whether disease control programs are well integrated into the general health system.[7, 8]

In the program implementation process, smooth implementation of the disease specific control program has often been hindered by challenges or bottle necks that exist in the health system. Through the effective intervention to these challenges/bottlenecks,expansion of the health programs and strengthening of the general health system are expected, bringing a synergistic effect on other disease specific programs and furthermore general health system.

This study was undertaken in order to assess the interface between disease specific programs supported by GFATM and HSS, with special reference to interaction between malaria control programs and general health systems.

Methods

The primary surveys were conducted for Viet Nam (2009) and Nepal (2012), by setting the National Malaria Control Programs supported by GFATM as entry points with special emphasis on good practices and challenges/ bottlenecks in implementing malaria control program in relation to the components of health system. Data from the two countries was updated as long as possible, based on information obtained by the supplemental survey during 2012-14 and by documents. In each country, the survey was conducted at various levels (from central to primary level) by document reviews, key informant interviews, and observation of facilities. The results of the primary surveys were analyzed, summarized in reports and submitted to WHO Western Pacific Regional Office.[9]

Document reviews

Documents related to health systems, National Malaria Control Programs (NMCP), health statistics, GFATM supports, etc. were collected and reviewed.[10-15]

Key informant interviews

Key informant interviews were conducted with the health staff at each level (central, regional, district, and community level) of the health facility, health managers at the district and provincial levels, and program managers of Ministry of Health (MOH in Viet Nam) or Ministry of Health and Population (MOHP in Nepal),WHO, NGOs, and other partner agencies. Leading contents of the interview included an outline of the health system, general information on malaria control programs in relation to the health system. Good practices and strengths to overcome challenges/bottlenecks, and on-going interventions for existing bottlenecks, integration between NMCP and general health system, etc., were also considered

Field surveys

In addition to surveying the capital cities of each country, malaria endemic areas, where Malaria Control Programs have been implemented with the support of GFATM, were selected (Thanh Hoa and Dien Bien Provinces in Viet Nam, and Dhanusha District and Hetauda City in Nepal). General information on health and medical care, information on health system and health program implementation, etc. were collected at Provincial Health Offices and District Health Offices, followed by surveys at health facilities at the primary level (or community level).

Analysis

Information obtained from the interviews, field surveys, and documents were summarized from the viewpoint of interface between malaria control programs and the general health system in accordance with the six building blocks (components) of a health system (Leadership and governance, Service delivery, Workforce, Information system, Medical products and technology, and Financing) proposed by WHO.[16]Good practices and bottlenecks/ challenges in the implementation of the control programs were identified, and possible solutions for the challenges/ bottlenecks were discussed.

Results

1. Overview of malaria conditions in Nepal and Viet Nam

In the two countries in this survey, Viet Nam and Nepal, malaria morbidity and mortality rates were considerably high in the past and malaria was given the highest priority in the health policy by the governments. Since the mid-1990s, malaria controls were actively implemented in these countries based on the National Malaria Control Programs (NMCPs) and the principles of Roll Back Malaria, which

consisted of strategic priorities including vector control and personal protection through the distribution of bednets (insecticide-treated bed nets; ITNs and long lasting insecticidal nets; LLINs), early diagnosis and prompt treatment (EDPT)including rapid diagnosis tests (RDTs) and artemis in combination therapies (ACTs), malaria surveillance and epidemic preparedness, behavioral change communication (BCC), and improvement in program management along with setting up targets for control. In particular, since the early 2000s, GFATM has contributed a large budget to malaria control programs (60-65% in Viet Namand70-78% in Nepal of the total malaria control budget). As a result, malaria in these countries has decreased remarkably in recent years, reaching pre-elimination levels.

2. Outline of general health systems in Nepal and Viet Nam

Health networks in the two target countries have been created at the central to commune level in accordance with the administrative strata (e.g. in Nepal; central—regional—district—commune levels,in Viet Nam; central—provincial—district—commune levels). Treatment care systems as well as preventive care systems have also been basically constructed in accordance with the administrative strata. (i.e. central, provincial (or regional) and district hospitals and commune health stations, etc.).A referral system, as well as health information and supply systems, function based on this network. In addition, some health control programs have their own systems (e.g. national institutes, provincial or regional control centers, district control centers).

Commune Health Stations (CHSs) provide health care at the primary level and under the CHSs, there are health posts (HPs) and sub-health posts (SHPs). These CHSs along with HPs and SHPs have the tasks of providing primary health care services, first-aid and treatment, implementation of national health programs, assisting in normal deliveries, family planning practices, health promotion, etc. There are commune (village) health workers in each commune, under the direct management and direction of the CHSs and commune leaders.

3. Characteristics of malaria control in relation to the health system

A: Characteristics of general health system (horizontal health system)

B: Characteristics of malaria control (vertical health system)

3-1 Nepal

Leadership and governance

A: The current long-term health plan (1997-2017) aims to provide health services throughout the country, particularly extending the primary health care system to the rural population and improving the health status of vulnerable populations, such as women and children, the rural population, the poor, the underprivileged and the marginalized. In recent years, the government has attached high importance to the promotion of education among residents and many primary schools have been constructed.

B: Since the launch of the Insect Borne Disease Control Program in 1954, the government has given malaria control high priority, and since 1993, the Epidemiology and Disease Control Division (EDCD) under MOHP has taken the lead in the malaria control program. Currently, the control program is basically implemented using the existing health system.

Service delivery

A: Efforts have been made to design a health system hierarchy from MOPH to the primary level, in order to ensure that the majority of the population has access to public health care facilities and can receive minor treatment at affordable prices. The government has strengthened the HPs and SHPs, which serve as first contact to basic health services and the venue for community-based activities with the support of GFATM. The pull system for essential drug supply was expanded to all 75 districts in 2010.

B: Delivery of LLINs is managed by the Population Service International (PSI).At local level, distribution is managed by NGOs/other partners and implementation is carried out by a broad range of community based organizations. Monitoring teams for ITNs and LLINs were organized at the district level. Mobile teams were organized, and are responsible for prevention, diagnosis and treatment, in endemic areas. RDTs and microscopy have become available as diagnostic tools for malaria by HPs due to the support of GFATM, anda cold chain for vaccination was used for storage of RDTs.

Workforce

A: Since the establishment of the first school of medicine in 1980, the number has markedly increased (22 schools of medicine in 2014). The increased number of medical doctors, nurses, and other co-medical staff has contributed to increased health and medical care of the Nepalese people.

B: Female Community Health Volunteers (FCHVs) were organized at the commune level for integration activities including malaria control. Training on malaria control for the health staff has been integrated with other disease control and training has been conducted with the support of GFATM at the peripheral level.

Information system

A: The routine monitoring system has been improved over the years. The Health Management Information System (HMIS), Logistics Management Information System (LMIS) and Fiscal Management Information System (FMIS) have also been well developed over the last 10 years. Health related activities are recorded and reported from the lowest health unit right on up to the district hospitals. In addition to HMIS, other individual programs are also providers of information.

B: Sentinel sites were set up for malaria outbreak surveillance. Reporting and monitoring systems in the public sector, from the peripheral level up to MOHP level, have been strengthened.

Medical products and technologies

A: In 2007, the National Drug Strategy was revised and the National Essential Drug List was established. In order to manage the above processes effectively,HMIS is used.

B: A considerable amount of ITNs, LLINs, RDTs, ACTs, and slide glasses for microscopic testing were provided at the peripheral level with the support of GFATM.

Financing

A: The amount of the budget for health programs funded by the government, and the percentage of the health budget within the total budget, were increased (from 5.1% in 1998 to 6.3% in 2013).

B: Since 2004, GFATM has greatly contributed to the prevention and treatment of malaria, particularly through the distribution of LLINs, ACTs and RDTs in high risk areas, along with the training of health workers and BCC activities for the residents. Treatment drugs are administered to patients free of charge at public medical facilities nationwide and diagnostic services for malaria are provided free of charge at all public sector health facilities in high endemic areas.

3-2Viet Nam

Leadership and governance

A: Since the 1990s the government has worked hard to strengthen the general health system. The international community has also cooperated with policy implementation at various levels. For National Health Programs, National Steering Committees are organized and programs are managed more intensively and efficiently with strong leadership and inter sectoral collaboration. Related major national institutes are responsible for the executive centers of the respective health programs, as well as the provision of technical advice, operational research and staff training.

B: The government attached highest priority to malaria control in all health programs. NMCP, which began in 1991, has been reinforced by the strong leadership of the Government and National Steering Committee, which consists of multi-sector members,utilizing the vertical malaria control system along with general health system. The general health system has also been strengthened and used as a malaria control program. Recently, high priority has been attached to control in frontier areas. A high literacy rate, effective use of school health education, education for residents in endemic areas, and preparation of guidelines have also facilitated the smooth implementation of the program.

Service delivery

A: The local administration is organized into provincial, district and communal political units which are responsible for the implementation of the health programs. All medicines and medical equipment are supplied by the government through the administrative strata. Participation by health facilities under the military, police and other sectors providing medical services to the population has helped increase health care coverage. The referral system among medical institutions was strengthened in collaboration with foreign assistant partners.

B: Service delivery in malaria control was basically carried out utilizing the general health system. The Army Medicine, People's Committee, Women's Union and other local organizations helped deliver bed nets and other services in the control program.

Workforce

A: Since the 1990s, human resources in health care have been trained both quantitatively and qualitatively. Improvement in training capacity to increase human resources has been observed. Commune health workers have participated in various programs.

B: The government has implemented a policy to train health workers for malaria control with the cooperation of foreign donors. The role of public organizations, such as women's unions and youth unions, in the

implementation of malaria control programs and collaboration with the military in hard-to-reach areas, are also noteworthy.

Information system

A: The reporting and information system functions efficiently, and reports from the primary level are transmitted to upper levels. The role of the mobile team is outstanding in information transmission and guidance in program implementation.

B: Reports on malaria from the primary level are transmitted to upper levels and then feedback is provided. Currently, considerable parts of these systems are integrated into the general health system. There are many examples of prompt and appropriate responses in cases of disease outbreak. The role of the mobile team is outstanding in the transmission of information and in the provision of guidance for program implementation.

Medical products and technologies

A: Development of the pharmaceutical industry has contributed considerably to the implementation of essential drug policies targeting primary health care. Drug quality is managed following the good practice criteria based on standards and guidelines for drug production, quality control, storage and distribution. Currently, all facilities are to follow the standards of Good Manufacturing Practice (GMP)-WHO.

B: Production of drugs used for malaria treatment, insecticides, and bed nets within the control program has gradually shifted to local sources and the drugs are supplied to the peripheral level under the proper guidance of the government. Widespread distribution of artemis in suppositories at the primary level has greatly contributed to a lower mortality rate.

Financing

A: A broad orientation of health financing was set in the 1990s through the development of health insurance, the partial user fee policy, and the Government's resolution on "social mobilization" in areas of education, health and culture. The government also focused on subsidies to users of health services, such as health care for the poor and children under 6 years of age. The health budget has continued to increase in line with the economic development of the country.

B: Initially, the governments ought to increase the malaria program budget, and the People's Committee of Viet Nam and international community financially supported the program. GFATM has greatly contributed to the expansion of the malaria program by strengthening activities for high risk groups.

4. Good practices in malaria control

Table 1 summarizes the good practices in the general health system which are regarded as having a good effect on malaria control as shown in table. Table 2 summarizes the good practices in the malaria control programs.

5. Bottlenecks/challenges

Table 1 Good practices in the general health system (Strengthening of General Health System), which contributed to malaria control

	Leadership and governance
1	Effort of the government to strengthen the general health system
2	Efficient management of the National Health Programs with strong leadership (V)
3	High priority given to the promotion of education
	Service delivery
4	Strengthening of health posts and sub-health posts
5	Effective service delivery in accordance with the administrative strata (V)
6	Support of military and police to service delivery (V)
7	Contribution of the mobile teams
8	Expansion of the pull system for essential drug supply
	Workforce
9	Increased training opportunities for health workers.
10	Marked increase of the number of doctors and nurses (N)
11	Improved skills by commune health workers conducting various programs
	Information system
12	Improvement in the routine monitoring system
13	Contribution by the mobile teams (V)
	Medical products and technologies
14	Management of drug quality based on the standards and guidelines (V)
15	Development of the pharmaceutical industry and its contribution to the essential drug policy (V)
16	Revision of the National Drug Strategy (N)
	Health financing
17	Substantial support from the GFATM
18	Increase funding of the health budget by the government
19	Development of the health insurance, partial user fee policy, subsidies to users of health services (V)

(V): Outstanding in Viet Nam, (N): Outstanding in Nepal

Table 2 Good practices in malaria control

	Leadership and governance
1	High priority of malaria control by the government
2	Strong leadership of the government and the National Steering Committee (V)
3	Utilization of the general health system in the malaria control program
	Service delivery
4	Participation by the Army Medicine, People's Committee, Women's Union and other local organizations(V)
5	Monitoring teams for ITNs and LLINs at the district level (N)
6	Contribution by the mobile teams
	Workforce
7	Increased training opportunities for health workers
8	Contribution bywomen's unions, youth unions and military in hard-to-reach areas(V)
9	Contribution by Female Community Health Workers (N)
	Information system
10	Monitoring visits and periodical submission of reports
11	Setting up the sentinel sites for malaria outbreak surveillance(N)
12	Appropriate transmission of the information and feedback to primary level (V)
	Medical products and technologies
13	Provision of considerable amount of ITNs, LLINs, RDTs, ACTs
14	Domestic production of treatment drugs, insecticides and bed nets(V)
	Health financing
15	Considerable financial supportfor malaria control by GFATM
16	Treatment drugs provided free of charge at public facilities

(V): Outstanding in Viet Nam, (N): Outstanding in Nepal

ITNs: insecticide-treated bed nets, LLINs: long lasting insecticidal nets,

RDTs: rapid diagnosis tests, ACTs: artemisinin combination therapies

Table 3 Existing challenges and bottlenecks in malaria control

	A. Leadership and governance	Viet Nam	Nepal
1	Weak program management capacity		++
2	Introduction of malaria associated with population movement	++	++
3	Weak health system in remote (frontier & border) areas	+	++
4	Weak coordinationbetween medical institutions, public-private sectors and laboratories	+	++
	B. Service delivery	**Viet Nam**	**Nepal**
5	Inequality in the distribution of bed nets (to vulnerable people)		++
6	Many hard-to-reach areas		++
7	Weak coordination between local government and GFATM in the distribution of bed nets	+	++
	C. Workforce	**Viet Nam**	**Nepal**
8	Shortage of health workers and manpower in remote areas		++
9	Low skill level of health workers in remote areas	++	+
10	Frequent changes in health workers and manpower in remote areas	+	++
11	Limited number of entomologists	+	++
	D. Information system	**Viet Nam**	**Nepal**
12	Weak private health sector		+
13	Poorly developed reporting system from the private health sector	+	++
14	Inadequate disease surveillance system	++	+
	E. Medical products and technologies	**Viet Nam**	**Nepal**
15	Inadequate quality assurance system for malaria testing	+	++
16	Weak function of the National Reference Laboratory		++
17	Difficulty in treatment due to increasing anti-malaria drug resistance ofP. Falciparum	++	++
	F. Health financing	**Viet Nam**	**Nepal**
18	Sustainable supply of health products (ACT, RDT, LLIN)	+	++
19	Low incentive for health workers	++	++
20	Heavy dependence on GFATM (sustainability is a challenge)	+	++
	Other	**Viet Nam**	**Nepal**
21	New endemic areas have been reported (environmental and social factors are suspect)	++	++
22	Increased number of cases of imported malaria	+	++
++: major challenges/bottlenecks, +: intermediate, No mark: minor			

Discussion

The general health system in Nepal used to be fragile in the past, but has gradually been strengthened and has been utilized in greater part in the malaria control program.[17]During the period of political instability (1996-2006), health systems were affected, but malaria control was minimally affected compared to other disease control programs due to its high governmental priority and the continuous support of the international community.

The Vietnamese government has worked hard to strengthen the existing health systems since the 1990s (both malaria specific and general health systems). The international community has also cooperated with policy implementation of Viet Nam at various health system levels.[18]Malaria control measures were effectively implemented under the strong leadership of the National Steering Committee, which has further strengthened and utilized the existing health system and mobilized public organizations.

Generally, collaboration among disease specific programs at upper levels is limited, and a health staff, as well as an infrastructure, is dedicated to each program. However, health care at lower levels has greater integration(both in Nepal and Viet Nam). By coordinating with community and social organizations, health workers carry out various tasks, such as primary health care, implementation of national health programs, preventive medicine, IEC activities, etc. GFATM and other assistant partners provide support by promoting training and supplying essential medicine and equipment. Malaria control has gradually become integrated with the primary health care system.

Best practices were identified from survey results. Among these, intensified education for residents focusing on disease prevention, strengthening of facilities at the primary level such as health posts and training health workers, utilization of health volunteers at the primary level, giving frontier areas high priority, and setting up mobile teams, were noteworthy and were held in in common by both countries. In addition, effective implementation under the strong leadership of the National Steering Committee could be seen in Viet Nam, utilization of the existing heath system was outstanding.

A synergetic effect of disease specific programs(vertical health programs) such as malaria control on the general health system could be seen to some degree, particularly at the primary level. The management system of vertical health programs appeared to have a good impact on the general health system at various levels. However, dissociation between vertical malaria control program and horizontal general health system also seems to exist. In addition, similar to other reports,coordination between malaria control programs and other disease specific programs is limited in many cases.[5,6] It is true that carrying out a vertical health system is important in implementing a malaria control program, but intensification of the malaria control program does not automatically lead to strengthening of the general health system.[19]More effort is needed to realize maximum synergy between disease specific programs and the general health system, as well as among different health programs.

As seen in the results of a similar study in Laos, if the general health system appears weak, a strong vertical health system supported by GFATM can function separately from the general health system.[20,21] These findings were also observed in Nepal and Viet Nam, particularly at the early stage of support by GFATM, where disease specific programs utilized procurement, information, monitoring systems, etc., outside of the MOH (MOHP), with varying levels of support and input provided by the disease specific divisions.

One of the current leading challenges/bottle necks is the limited coverage and quality of malaria control measures among populations living in remote areas. Most health workers are concentrated in large cities and towns, while the health personnel and/or medical supplies of many health facilities at the primary level and at some district hospitals are still insufficient. A poorly developed reporting system from the private health sector, inadequate quality assurance system for malaria testing, and weak coordination between the local government and GFATM in the distribution of bed nets were also pointed out (particularly in Nepal). In Nepal it was suggested that bed nets are not always distributed to the vulnerable populations, as similarly reported in some African countries.[22, 23]In addition, the current heavy dependence on GFATM undermines the assurance of sustainable malaria control.

Introduction of malaria due to population movement, increased drug resistant malaria, and the emergence of new endemic areas have become growing issues in malaria control in recent years in many endemic countries. Although not always health system related, these issues do affect health systems and active health system strengthening seems to be crucial for their control. Such growing challenges are often related to political issues, poverty, and a changing environment due to indiscriminate development, global warming, etc.[24,25] In order to address these growing challenges, strong government leadership, a sector-wide approach, and inter sectoral collaboration are required.

It is crucial to implement effective malaria control programs which address these challenges and bottlenecks, seeking their elimination. Particularly, emphasis on strengthening the health systems in remote areas, training of the health staff at the peripheral level, diagnosis based on accurate quality assurance, promotion of public-private relationship and addressing the issue of imported malaria, are desired. To create sustainable health systems, serious consideration of issues regarding the availability of domestic resources, including workers, supplies and local participation, as well as budgetary resources, are needed. Moreover, good practices which have been identified in this survey are expected to provide useful lessons in the effective implementation of malaria control in endemic countries. Addressing these issues will directly lead to further strengthening of the health systems and eventually to the effective implementation of various health programs.

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