

**2010 Nagoya-Yonsei University  
Research Exchange Meetings on Health Sciences**



**October 23, 2010  
Auditorium at East building  
Daiko Campus  
Nagoya University  
Nagoya, Japan**

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## Preface

It is my great pleasure to host the 2010 Nagoya-Yonsei University Research Exchange Meeting on Health Sciences (October 22-23, 2010) at the Daiko Campus in Nagoya University. This is the first meeting that is held to promote academic exchanges between Nagoya University, School of Health Sciences and Yonsei University, College of Health Sciences and College of Nursing in Korea.

This booklet shows presentation contents of the research activities of the graduate students and faculty members of both universities in the research exchange meeting. Each of these presentations expresses the high research activities in health sciences.

Needless to say, the recent globalization progresses very fast in every field, and our activities, the scientific research and the education in the university, have also undergone a big change. Although health sciences are still in a relatively young science fields, it will be a chance for us to create and develop new health sciences with the advance of the globalization. We, academics in the fields of the health sciences, need to challenge to create a future-oriented healthcare research for the future society.

Nagoya University is one of the leading universities in Japan and Yonsei University is the oldest private university and a leading institution of higher education and research in Korea. As a leading University, we are convinced that this research exchange meeting will make great contribution for the development of both universities in the future.

Next research exchange meeting will be held at the Yonsei University in 2011 in Korea. We hope that the interchange between both universities of the research and education activities will develop more in the near future.



Hisataka Sakakibara  
Director  
Nagoya University, School of Health Sciences

## 2010 Nagoya-Yonsei University Research Exchange Meetings on Health Sciences

### Organizing Committee

#### **Chair:**

Hisataka Sakakibara

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Sumio Yamada

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Chiyo Kawaura

Jun Ueyama

Jun-ichi Uemura

Kazuteru Niinomi

Naoko Ito

## CONFERENCE SCHEDULE

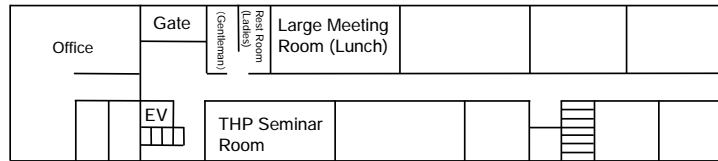
Symposium Venue: Nagoya University School of Health Science  
Banquet: Soleil in Nagoya University Hospital

DATE	SCHEDULE	
Oct. 22 (Fri.)	14:20	Arrival at Centrair Airport
	16:30	Hotel Check-in
	17:45 – 18:45	Nagoya University Hospital Tour
	19:00 – 22:30	Banquet (Soleil in Nagoya University Hospital)
	21:00	Back to Hotel
Oct. 23 (Sat.)	9:45 – 10:00	Opening Remarks (Auditorium in East Building)
	10:00 – 11:20	Keynote Address (Auditorium in East Building)
	11:30 – 12:10	Session in Health Administration (Auditorium in East Building)
	12:10 – 13:30	Lunch (Large Meeting Room in Main Building)
	13:30 – 16:00	Subcommittee Sessions
		Medical Technology (THP Seminar Room in Main Building)
		Radiological Technology (Room2 in Main Building)
	Physical Therapy (Room3 in Main Building)	
	Occupational Therapy (Room4 in Main Building)	
	Nursing (Room1 in Main Building)	
	Dinner ( <i>in each major</i> )	
Oct. 24 (Sun.)	10:00 –	Sightseeing
	15:20	Departure for Seoul

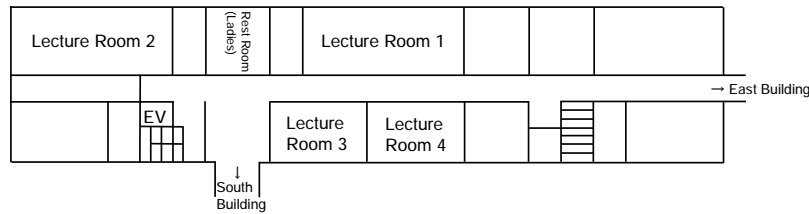
Campus map



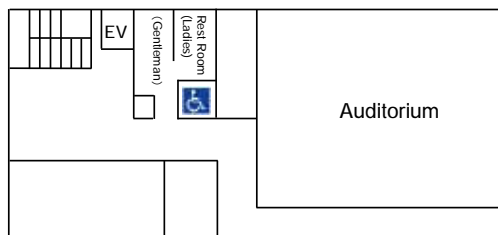
- 1 School of Health Sciences (South Building)  
Daiko Medical Center
- 2 School of Health Sciences (Main Building)
- 3 Gymnasium
- 4 Annex to Radioisotope Laboratory
- 5 Energy Center
- 6 Student Hall
- 7 Garage
- 8 Annex to School of Health Sciences
- 9 Kyudo (Japanese Archery) Hall
- 10 School of Health Sciences (East Building)
- 11 Researchers Village Daiko



Main Building 1F



Main Building 2F



East Building 4F

**Keynote Address (10:00 ~ 11:20 Auditorium of East Building)**

Session chairs: Prof. Sumio Yamada, Prof. Tetsuhito Kojima

**KA-1 "Present and Future of Health Science Research in Japan"**

Prof. Yoshie Kodera, Univ. of Nagoya, JAPAN

**KA-2 "The Present and Future of Korean Healthcare Industry"**

Prof. Youngjoon Seo, Univ. of Yonsei, KOREA

**Session-Health Administration (11:30 ~ 12:10 Auditorium of East Building)**

Session chair: Prof. Takaaki Kondo

**HA-1. "Quality Assessment of Stroke Treatment and Pay-for- Performance in Japan and Korea"**

Seungwon Jeong (Health Scientist)

**HA-2. "Total Health Expenditure and Its Structure in Two Asian OECD Countries, Japan and Korea"**

Jung-Woo Shin (D)

**Session-Nursing (13:30 ~ 16:00 Lecture Room 1)**

Session chairs: Prof. Makoto Hirai, Prof Midori Asano

**N-1. "Health Status and Health Care Utilization by Marriage-based Women Immigrants in Korea and Policy Directions: Focus on Mental Health"**

Yang-Heui Ahn (Professor)

**N-2. "Association of Underweight with Low Lymphocyte Count and Prealbumin as Indicators of Malnutrition in Japanese Women"**

Tomoko Nishida (Aichi Health Plaza), Yukiko Okamura, Hisataka Sakakibara

**N-3. "Comparison of Salivary Cortisol, Heart Rate, and Oxygen Saturation between Early Mother-Infant Skin-to-skin Contact Methods with Different Initiation and Duration Times in Healthy, Full-term Infants."**

Yuki Takahashi (D), Koji Tamakoshi

N-4. "Evidence-Based Nursing Strategy for Acute Ischemic Stroke Patients Management in the Emergency Department"

Ji-Eun Baek (M)

N-5. "Development of a Japanese Functional Health Literacy Test"

Katsuyuki Nakagami (D), Toyoaki Yamauchi

N-6. "Related Factors of Self-Efficacy in Cancer Outpatients Receiving Chemotherapy in the National University Hospital"

Akiko Hayashi (Konan Kosei Hospital), Shoko Ando

#### Session-Radiological Technology (13:30 - 16:10 Lecture Room 2)

Session chairs: Prof. Yoshie Kodera (RT1-4), Associate Prof. Masatoshi Tsuzaka (RT5-8)

RT-1. "Educational system for radiological technologists in Japan

- As the self-introduction of our department and Japan -"

Megumi Watanabe (M)

RT-2. "Advancement of Medical Imaging and Instrumentation in Radiological Science"

Hee-Joung Kim (Professor)

RT-3. "Noninvasive Estimation of Regional Cerebral Blood Flow using <sup>123</sup>I-IMP Acquisition Data"

Shinji Abe (D), Katsuhiko Kato, Yoshitake Takahashi, Masato Yamashita, Naotoshi Fujita, Naotoshi Ohta, Yasukazu Kajita, Shinji Naganawa

RT-4. "An Influence of the Beam Quality on the Noise of the Image in Digital Mammography"

Maki Yamada (M), Yuri Kato, Naotoshi Fujita, Yoshie Kodera

RT-5. "Multi-material Decomposition Imaging in the Photon Counting Detector"

Hyo-Min Cho (D), Yu-Na Choi, Hee-Joung Kim

RT-6. "Dosimetric Characterization of Flattening Filter Free Linac using Monte Carlo Simulation"

Takuma Matsunaga (M), Tomohiro Shimozato, Yuichi Aoyama, Hiroshi Fukuma, Ryuichi Yada, Hiraku Fuse, Yusuke Oribe, Masataka Komori, Yasunori Obata

RT-7. "Estimation of Radiation Doses from x-ray CT using Monte Carlo Simulation"

Yuki Morishita (M), Shuji Koyama

RT-8. "Evaluation of the Distribution of Intracranial Navigation Accuracy in Image-guided Neurosurgery"

Takashi Mitsui (M), Masatoshi Tsuzaka, Yuichiro Hayashi, Yoshinori Asahina,

Kentarō Sugiura, Masazumi Fujii, Toshihiko Wakabayashi

#### Session-Medical Technology (13:30 - 16:00 THP Seminar Room)

Session chairs: Prof. Shinya Wakusawa, Prof. Tsutomu Kawabe

MT-1. "Sleep Disorder and Sleep-disordered Breathing in the Elderly"

Chie Nakazaki (M), Akiko Noda, Nobuko Fujita, Sumio Yamada, Yasuo Koike, Shinya Wakusawa

MT-2. "Mycobacterium Tuberculosis-induced Expression of Granulocyte-Macrophage Colony Stimulating Factor is Mediated by Erk1/2 MAPK or PI3-K/Akt Signaling Pathway"

Sung-Jung Park (D), Hye-Young Lee

MT-3. "Molecular Basis of Decrease of Protein S in Pregnancy: 17 $\beta$ -estradiol Down-regulates Protein S (PS) Expression Recruiting Co-repressor Complex to the PS Promoter"

Atsuo Suzuki (D), Yuhri Miyawaki, Junko Fujita, Asuka Maki, Yuta Fujimori, Akira Takagi, Takashi Murate, Hidehiko Saito, Tetsuhito Kojima

MT-4. "Comparison of REBA HPV-ID with DNA Chip Based Assay for HPV Genotyping"

Sung-Hyun Kim (D)

MT-5. "Morphometric and FISH Analyses of Adenocarcinoma and Mesothelial Cells Using Liquid-Based Cytology"

Ayumi Morimoto (D), Tetsuro Nagasaka, Katsunori Hashimoto, Toyoharu Yokoi

MT-6. "Molecular Mechanism of Ceramide Kinase Gene Repression on ATRA-induced Neuronal Differentiation"

Masashi Murakami (PD), Hiromi Ito, Kazumi Hagiwara, Noriko Sasaki, Misa Kobayashi, Asuka Hoshikawa, Akira Takagi, Tetsuhito Kojima, Keiko Tamiya-Koizumi, Yoshiko Banno, Yoshinori Nozawa, Takashi Murate

#### Session-Physical Therapy (13:30 - 16:00 Lecture Room 3)

Chaired by the academic adviser of each presenter

PT-1. "Development of an Instrument for Measuring Patellar Mobility and its Clinical Application"

Susumu Ota (Assistant Professor), Morio Kawamura

PT-2. "CD105 Positive Mesenchymal Stem Cells from Mouse ES Cells Show High Potential for Differentiation"

Nana Ninagawa (D), Eri Isobe, Rumi Murakami, Shigeko Torihashi

PT-3. "Effects of Forced Impaired Forelimb Use on Central Nervous System after Small Internal Capsule Hemorrhage in Rats"

Akimasa Ishida (D), Keigo Tamakoshi, Michiru Hamakawa, Hiroki Nakashima, Haruka Shimada,  
Hideki Hida, Kazuto Ishida

PT-4. *"Recurrent Risks after Mild Ischemic Stroke in a Japanese Population"*

Yuji Kono (D), Sumio Yamada, Kenta Kamisaka, Amane Araki, Yusuke Fujioka, Keizo Yasui,  
Yasuhiro Hasegawa, Yasuo Koike

PT-5. *"Locomotor Imagery Training Improves Gait Performance in People with Chronic Hemiparetic  
Stroke"*

Hye-Seon Jeon (Professor)

PT-6. *"The Effects of an Isometric Knee Extension with Hip Adduction (KEWHA) Exercise on  
Selective VMO Muscle Strengthening"*

Bo-ram Choi

#### Session-Occupational Therapy (13:30 - 16:00 Lecture Room 4)

Invited Lecture

Session chair: Prof. Yuji Sawada

IL-1. *"Occupational Therapy in Japan"*

Emi Ito (Associate Prof.)

IL-2. *"The Beginning and Development in the Field of Occupational Therapy in Korea"*

Eun Young Yoo (Professor)

Research Presentation

Session chairs: Prof. Eun Young Yoo, Prof. Kunifumi Suzuki

OT-1. *"Biological Observation during the Daytime of Elderly Patients with Advanced Dementia"*

Kaori Yamaguchi (D)

OT-2. *"Occupational Therapy as a Career: Korean Undergraduate OT Students' Preferences and  
Concerns Regarding Their Future as Occupational Therapists"*

Joo-Hyun Lee (M), Eun-Young Yoo

OT-3. *"Alteration of Time-perception in Young and Elderly People during Tasks"*

Yuko Iwamoto (D)

OT-4. *"Use of Scheduled Exercise Program Along With Forced Use to Increase the Function of Upper  
Extremity in Individuals with Hemiplegia"*

Hae-Yean Park (D)

Keynote Address (10:00 ~11:20 Auditorium of East Building)

Session chairs: Prof. Sumio Yamada  
Prof. Tetsuhito Kojima

## THE PRESENT AND FUTURE OF KOREAN HEALTHCARE INDUSTRY

Young-Joon Seo<sup>1)</sup>

1) Department of Health administration, College of Health Sciences, Yonsei University, Kangwon-do, Korea

### The present of Korean healthcare industry

Healthcare industry has been understood as a broad concept which includes pharmacy and healthcare appliances as well as healthcare services together with a narrow idea which refers to only healthcare service industry. When healthcare industry is defined as healthcare service industry, the recent argument of healthcare industry advancement in Korea has been mainly focused on changing the healthcare paradigm from a regulated healthcare system to a free market system such as deregulation, allowing for-profit hospital corporations and vitalization of a private health insurance market without having a clear goal or seeking the ideal model. Advancement of healthcare industry from the stance of the hospital which is playing an important role specially in the healthcare provider group has implicated that the health-related systems and policies should be established to contribute to the national economy through attracting overseas patients and creating employment as a highly profitable industry by developing an advanced healthcare technology and human resources. Furthermore, the individual hospitals internally feel they need to enhance competitiveness by improving all of the hardware and software systems as a world-class.

Competitiveness of Korean healthcare industry has been told to be weak compared to the international competitors even though clinical technology reaches world class in many areas. We can find the reason in the following several aspects. First, Korean healthcare industry have difficulties in marketing because there are a lot of limitations such as price control, regulation of advertising, etc. Second, by serving the large number of patients for a low margin of profit due to low medical fee, it is difficult for hospitals to care each patient delicately to meet their personal needs. Third, because financial resources of the healthcare industry mainly consist of public health insurance, supplementary private insurance and out-of-pocket money, the scale of domestic healthcare market is limited and it is difficult for healthcare providers to invest boldly in the clinical research and high-tech medical services (market scale of current domestic healthcare service industry is 5.6% in GDP, compared to about 10% of the advanced countries). Fourth, because the scale of medical-related support industry such as pharmaceutical and life sciences is small and its quality is not high, it does not exert a synergistic effect as neighbor industries.

### The prospects of Korean healthcare industry

There are several factors that can have a significant effect on future Korean healthcare industry. First, due to rapid aging population, demands for health and aged care, and health promotion (estimated \$70billion market in 2010) will dramatically increase and result in heavy financial burden to the individual household as well as the government. Second, the development of information technology and life sciences will bring the emergence of medical complex combined with BT, IT, pharmacy, and also make it easier to access to the U-healthcare. Third, the change of reimbursement system from fee-for-service to capitation and prospective payment system will drive hospital administrators to emphasizing more cost-effective management. Fourth, demands for the environmentally-friendly healthcare will be grown in the care

delivery process as well as hospital construction due to the global warming, resources depletion, air pollution, etc. Fifth, the quality assessment of healthcare that eventually converts into accreditation system will be more reinforced, and most of the hospital information will be released to the consumers and insurers to use as a selection criteria. Finally, facing a turbulent environment caused by kin competition among healthcare institutions, introduction of for-profit hospital corporations, growth of private health insurance market, and performance-based payment from third part payers, healthcare institutions will try to recruit and maintain high quality staff and to implement diverse management strategy.

### Future strategies of Korean hospitals

Korean hospitals have made various efforts so far in order to survive under the turbulent environment of healthcare market. However, it is true that many hospitals have still followed physician-centered traditional hospital management, and that operating systems of the hospitals are also far from those of advanced countries. In order for the Korean healthcare system and hospitals to be advanced, there are some national tasks to be solved. First, the government has to build advanced healthcare system that all the people can receive good quality of care without worrying about medical bills by increasing public insurance coverage dramatically from the current 65% to 90% level. Second, the government should change the paradigm that has focused on disease treatment to the disease prevention and health promotion. Third, Integrated Delivery System (IDS) which is defined as a network of organizations that provides a coordinated continuum of care to a defined population should be established in order to deliver appropriate care to the people at right time at right place. Fourth, the government should actively foster the complex healthcare industry which bundles up BT, IT, pharmacy, and business. Fifth, the government should free unnecessary regulation that has restrained the hospital's autonomy and creativity, while intensify the hospital's responsibility for patient's safety and environmentally-friendly care. In addition to the government role, hospitals themselves also have to make efforts to improve their internal effectiveness. First, hospitals should build customer-oriented care delivery system through restructuring and reengineering of the hospital. Second, hospitals should generate synergy effect through networking with other healthcare institutions - vertical and horizontal integration, and integration of oriental-western medicines, etc. Third, customer-centered marketing should be strengthened. To do this, hospitals build customer database and should strengthen the formal and informal contacts with target customer groups. Fourth, hospitals should establish the culture of labor-management cooperation by improving the quality of workers' life and open management. Fifth, hospitals should provide a personalized patient-centered care through case management system. Finally, hospitals should strengthen the relationship with the local communities through formal and informal communication and economic contribution to the community.

### Conclusion

The advancement of the healthcare industry in Korea could be accomplished when government, consumers and providers



agree on what the most desirable healthcare system in Korea should be in the future and how it is achieved. It means that the true advanced health care system will be created when all the stakeholders in healthcare market clearly recognize their rights and responsibility, and extend mutual understanding and cooperation with sharing the goal of 'Healthy and Welfare State of Korea'.

Health Administration (11:30 ~12:10 Auditorium of East Building)

Session chair: Prof. Takaaki Kondo

## Quality Assessment of Stroke Treatment and Pay-for- Performance in Japan and Korea

S. Jeong<sup>1)</sup>

1) Center for Well-being and Society, Nihon Fukushi University, Nagoya, Japan

### Introduction

P4P (Pay for Performance) is considered to be a tool that can enhance health care quality and improvement. Reports show that P4P has been increasingly used in developed countries such as the United States<sup>1</sup> and the United Kingdom.<sup>2</sup> Nevertheless there is a lack of reports on formal evaluation of hospital P4P in the literature. Systematic evaluation of hospital P4P is needed to understand the effect and benefits of investing in P4P.<sup>3</sup>

Similar P4P programs are under way in Korea and Japan, too. This study reviews the status and examines the performance in the two countries.

### P4P in Korea

In Korea, The cause-specific death rate by stroke and cerebrovascular diseases in recent years has been on the decline, but stroke is still ranked the number one disease killer, accounting for the highest percentage of the global burden of disease such as disability combined and therefore causing increased medical costs. In consideration of this, an acute stroke assessment tool that has been developed to provide data to care hospitals for promoting their voluntary quality improvement activities has been used since 2005. Also, the 2006 survey on the status on the medical services for stroke patients was conducted for the similar purpose. Based on these results, a pilot P4P project for acute stroke was undertaken from 2007 throughout 2010.

Among the assessment criteria were Documentation of smoking history, Neurologic exam.rate, Screen for dysphasia by end of hospital day two, brain scan within 24 hours of admission, Lipid Profile, T-PA considered, Antithrombotic medication within 48 hours of admission, Discharged on Antithrombotic, Patients with Atrial Fibrillation Receiving Anticoagulation therapy.

### P4P in Japan

There have been growing concerns and requirements for health care quality in Japan. The introduction and implementation of P4P (Pay for performance) programs for rehabilitation and recovery in 2008 was a significant advancement in the area of health care service. However, there still remains a gap involving a need for close examination of what factors may critically influence the rehabilitation medical service quality and the degree to which hospitals have made or will make efforts in achieving best practice in rehabilitation. To begin with, the assessment criteria for P4P set forth by the Japanese government are summarized as follows.

In Japan, P4P inpatient rehabilitation among stroke survivors began in 2008 with a primary objective to provide the services needed and improve post stroke patient functional recovery. Despite concerns over the effectiveness and quality of P4P by some stakeholders of the program in Japan,<sup>1</sup> three standards were developed for the P4P inpatient rehabilitation program. They included: (1) >60% of participating stroke survivors should be discharged to the community; (2) >15% of newly hospitalized patients should have severe stroke; and (3)

>30% of patients under the P4P program should demonstrate an improvement in their daily living functions or functional recovery at the time of hospital discharge.

### Result

#### Korea

The follow-up assessment for the two treatment periods, in 2005 and October to December of 2008, in the categories of 1) promptness in initial diagnosis, 2) responsiveness in initial treatment, 3) secondary preventive measures, and 4) initial assessment of patient conditions, revealed that there were overall improvement in quality compared to the results on 2005, but significant variations in treatment practices across types and care hospitals were observed (Figure 1 and 2).

Following this, the third assessment was conducted on the treatment period of January to March of 2010 with a goal of seeking for ways to reduce the discrepancies between hospitals and ensuring enhanced medical service quality for stroke patients. The numbers of subject hospitals were 187 for 2005, 194 for 2008, and 313 for 2010.

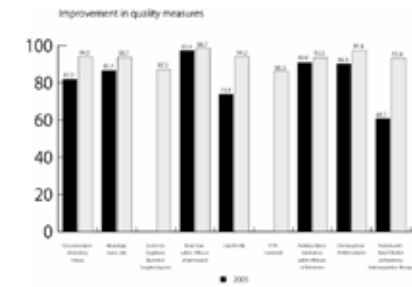


Figure 1. Improvement in quality measures

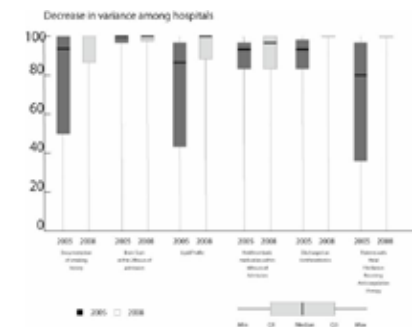


Figure 2. Decrease in variance among hospitals in Korea

Japan

The comparison of the data before and after the introduction of P4P showed that for FIM during hospital stay, the score after P4P (68.85) was slightly higher than that before P4P (65.82) without any statistical significance. On the other hand, the Rankin Scale score was found to be lower after P4P (3.28) than before P4P (3.78), showing higher scores for patients with light disability ( $p < .001$ ). With respect to FIM at hospital discharge, the scores were not significantly different between before (90.46) and after (91.86) P4P. Also, in the case of patients whose actual FIM was deteriorated from estimated FIM, the percentages were about the same with 34.8% and 34.4% for before and after P4P, respectively. The home return rate was 80.0% after P4P, compared to 73.0% before P4P, showing an increase of 7.0% point ( $p < .05$ ) (table 1).

Also the therapeutic results were compared between hospitals. We estimated the expected value of levels of ADL at discharge for rehabilitation patients using regression analysis. Furthermore, we identified the distribution of the expected improvement levels in ADL by hospitals.

The results showed large differences between hospitals (Figure 3).

Table 1. State of patients before and after P4P in Japan

State of patients	Before P4P 2006 & 2008.3 (N=205)		After P4P 2006 & 2008.3 (N=205)		P
	Mean	SD	Mean	SD	
Discharge rate	57.9	0.44	58.0	0.46	$P > .05$
Age	67.02	12.43	67.02	14.27	n.s.
# of days since onset	22.27	27.49	27.88	39.20	n.s.
Rankin Scale at hospital admission	3.78	1.03	3.28	1.24	$P < .001$
FIM at hospital admission	65.82	14.29	68.85	12.07	n.s.
FIM at hospital discharge	90.46	12.96	91.86	12.56	n.s.
Length of stay (in days)	92.91	49.09	94.09	47.09	n.s.
PT measure (Obs.)	40.94		40.28		71.77
OT measure (Obs.)	61.37	17.40	70.48	44.85	$P < .05$
ST measure (Obs.)	61.90	24.40	68.23	40.94	$P < .05$

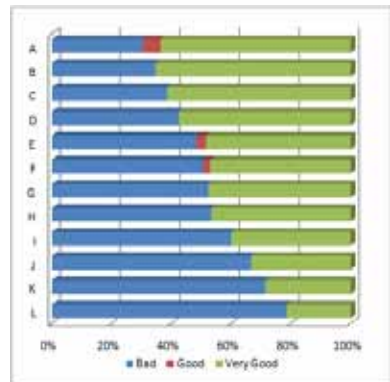


Figure 3. Decrease in variance among hospitals in Japan

Discussion

Many reports suggest that improvement in Korean medical services has been confirmed over the course of the three years

of the P4P pilot project implemented in Korea. However the judgment of the quality of medical services can vary depending on which criteria are used or which compound values are taken into account 4. This holds for the case of Japan.

The study in Japan focused on patients in recovery in the rehabilitation ward using a sample of 680 patients from 12 different hospitals after adjusting for triage at admission obtained from the databank (issued in September, 2009) in Japan and compared the therapeutic results between hospitals. The comparison found that there were statistically significant differences in the results of therapeutic achievement in each hospital.

Thanks to advances in the assessment of health care quality and wider publication of results from studies conducted in a number of first world countries such as the US and the UK, it is expected that health care providers will put a voluntary effort to improve their future health care services. Nonetheless, prior to such efforts of medical providers, it is essential to develop appropriate criteria for assessing health care quality and verifying the validity.

Conclusion

Donabedian (1980)5 mentions that those who have no experience with the complicated system of medical practice, are likely to think that assessing the quality of medical services would be as easy as weighing a bag of potato chips, but in the actual settings the task is not so easy as it appears. In quality assessment of medical services quality, there are not a few elements involved. Indeed, it should be insured that in designing the structure and process for targeted outcomes, the relevance to the therapeutic objective, the validity of the causality, the sensibility, the appropriateness of timing, costs, and the patient satisfaction, among others, are properly taken into consideration. Since these requirements are also applied to P4P, putting P4P in right place will be a rewarding but also challenging task.

References

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- [2] Doran T, Fullwood C, Gravelle H, et al. Pay-for-performance programs in family practices in the United Kingdom. *N Engl J Med*. 2006;355(4):375-384.
- [3] Mehrotra A, Damberg CL, Sorbero ME, Teles SS. Pay for performance in the hospital setting: what is the state of the evidence? *Am J Med Qual*. 2009;24(1):19-28.
- [4] S. Jeong, K. Kondo, N. Shiraiishi, Y. Inoue : An evaluation of the quality of post-stroke rehabilitation in Japan. *Clinical Audit* 2:59-66, 2010
- [5] A. Donabedian : Exploration in Quality Assessment and Monitoring: The definition of Quality and Approaches to Its Management. (1) . Health Administration Press, 1980.

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Total Health Expenditure and its structure in two Asian OECD countries, Japan and Korea

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Introduction

Improvement of economic and progress of aging increase not only a personal concern but also social interest about health. Beyond this, it is required to make a rational policy for operating a health care system efficiently and allocating the resources at the national level. However, to fulfill those needs, it is necessary to build a statistical infrastructure and health spending is regarded as one of the most important statistics. Total Health Expenditure(hereafter THE) is a core index showing health spending and a National Health Accounts(hereafter NHA) gives us clear information of tendency of THE.

The NHA does clearly organize the flow of health spending by tri-axial(Health care functions, Health care service provider industries and Sources of financing health care). The NHA is proceeding according to the SHA(System of Health Accounts, hereafter SHA) guideline published by Organisation for Economic Co-operation and Development (hereafter OECD) in 2000. Each economy collects the data and implements SHA in order to develop comprehensive and enhance a consistency. Moreover, this concept is based on the consensus on what element should be included or not, so the result is to ensure the comparability of health care expenditure estimated by different nation.

Those three kinds of element(Comprehensiveness, Comparability and Consistency) could be a strength and a main goal of SHA. Recently, OECD, EROSTAT and WHO have collaborated in the joint OECD-EROSTAT-WHO Health Accounts Data collection, they have also been engaged in projects to develop a methodology. Nevertheless, there are lots of problems harmonizing the criteria in several aspects such as an experience, a limitation of data and a cultural difference.

The paper begins by presenting the meaning of the SHA briefly which is used for collecting a data internationally, and examining the structure of THE in two countries, Japan and Korea. Additionally, this paper aims to provide a detailed review of THE in both countries comparing with other OECD economies.

A concepts and development of SHA

As noted above, The System of Health Accounts(SHA) proposes an integrated system of comprehensive and internationally comparable accounts and provides a uniform framework of basic accounting rules and a set of standard tables for reporting health expenditure data(Orosz and Morgan, 2004). It proposed in three dimensions, ICHA-HF, ICHA-HC and ICHA-HP. Firstly, ICHA-HF is related to financing sources. THE mainly comprises public financing(HF.1.) and private financing(HF.2.), and its composition determines the characteristics of health care system. Secondly, ICHA-HC is a classification of Function (e.g. Inpatient, Outpatient) which is the crucial element of the SHA. Consequently, health policy-making refers to it on the national level. Lastly, ICHA-HP shows information of providers: hospitals, nursing and residential care facilities etc.

The SHA has two major aims; one is to provide a framework for international data collection and the other is to suggest a possible model for redesigning and complementing

NHA to aid policy-makers(Jeong, 2010). To achieve this, a lot of health accounts expert meeting have been holding since 1990s and 'A system of Health Accounts' (version 1.0) was published by OECD in 2000. During the last decade, most of the OECD economies are able to implement basic tri-axial(ICHHA-HF, ICHA-HC, ICHA-HP) according to this manual. There was a demand for systemizing accounting system among developing countries, and the World Health Organization(WHO) published NHA Producers Guide to response those needs in 2003. Since then, a number of OECD and non-OECD countries have undergone SHA framework and have provide a data regarding health spending. In 2004, three international organizations build a cooperative system for harmonizing National Health Accounts and collecting a standardized health statistics. Such interests in Health Accounts are also spreading to across Asian-Pacific countries like Sri Lanka, India and China etc. Recently, the 6th APNHAN (Asia-Pacific National Health Account Network) expert meeting was held in Korea in last July 2010 and more than 50 experts participated from approximately 20 economies. As a result, the SHA manual(OECD, 2000) is used in a large and growing number of OECD and non-OECD countries as the standard accounting framework for statistics on health expenditure and financing.

A structure of THE in Japan and Korea

Prior to comparing the major findings of THE, it is required to close look at the characteristics of the structure of health spending and the data sources. Although many OECD and non-OECD economies are currently undertaking the OECD/SHA manual, some countries(including Japan) continuously use domestic standard. However, this makes lots of confusions.

In Japan, there are two types of representative Index showing health spending statistics, one is Japanese National Medical Expenditure(Kokuminryohi, hereafter NME) published by 'Department of Statistics and Information(Japanese Ministry of Health, Labor and Welfare)', and the other is OECD's Total Health Expenditure(THE) estimated by 'Institute for Health Economics and Policy(Japan, IHEP)'.(Jeong, 2001) The former is the total medical payment limiting its scope to medical service covered by the health insurance scheme, while the latter includes not only NME statistics but also other items like OTC drugs, health promotion services, Japanese long-term insurance(Kaigo Hoken), administrative finances and capital formation etc. In other words, the NME is a subset of THE. In considering the health spending to GDP ratio, there is a 2.6%p gap between THE(8.1%) and NME(6.5%) in 2007. In the case of THE, it is based on the number of statistics(around 40 kinds of statistical data) including NME statistics.(Mitsutake, 2010)

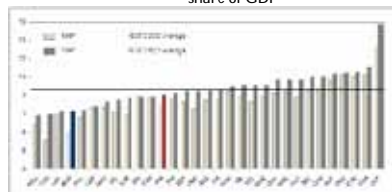
In Korea, estimation of health expenditure was attempted since 1976. Initially, many scholars tried various methods, but OECD's concept was accepted right after joining the OECD in the late 1996. Eventually, SHA-based health accounts have been reported annually by Jeong(focal point designated of OECD-WHO-EROSTAT) since 2004. Recently, a Health Accounts Forum was organized to spread its concepts and to

develop a method. For calculating THE, a variety of sources can be used individually or in combination.

A comparative analysis of THE in Japan and Korea

Total Health Expenditure accounted for 8.1% of GDP in Japan, slightly less than the OECD average(8.6%) in 2007. Although it increased 15.7% in the past 10 years, that is still below the OECD level. Meanwhile, Korea increased 53.7%(annually 10.4%) at the same period and it reached to 6.3% of GDP in 2007, even though it ranks(28<sup>th</sup>) at the bottom of OECD members. The United States is the country which spends tremendous amount of money on healthcare with 15.7% of GDP in 2007. Besides, 6 countries like France, Switzerland, Germany, Austria, Canada and Belgium followed with more than 10% of their GDP. Overall, health spending as a share of GDP has been increased during this period, but both of two countries level is significantly lower than the OECD average.(Figure1)

Figure1. Total Health Expenditure in Japan and Korea as a share of GDP



1) Source: OECD Health Data 2010(2010)  
2) Excluding Luxembourg and Portugal. (2007 not available data)

As shown by Figure2, most of the OECD countries, there is generally close agreement on the THE mainly financed by public sector with 71.2%. In Japan, 81.9% of THE is funded by public side in 2007 and this figure is almost similar to 10 years ago(81.5%). On the other hand, the share of public spending in Korea is obviously lower than that of OECD economies except the USA and Mexico. Although Social security scheme and government(public part) play an important role in Japan and Korea, there is a big different composition in financing sources.

Figure2. Share of THE by financing agent



1) Source: OECD Health Data 2010(2010)  
2) Excluding Luxembourg and Portugal. (2007 not available data)

Table1 gives details of health spending by Function in Japan and Korea. The allocation of health spending across the various service and medical goods shows the characteristics of health care system. The table shows that Korea and Japan report a relatively low proportion of expenditure for in-patient services(29.4%, 38.3% respectively) comparing to OECD average level(55.6%). On the other hand, spending for In-patient and Medical goods is high. Furthermore, large differences remain between Korea and Japan in Long-term nursing care. The ratio of Long-term nursing care accounted to

15.7% in Japan, notably higher than Korea(1.7%) where care tend to be provided in informal sector such as family.

In terms of hospital expenditure, out-patient care is around 27.5% of current health expenditure in Korea where hospitals maintain large out-patient department.(excluding a table because of restricted paper)

Table1. Total public and private health expenditure by function (US\$ PPP Per capita)

	Japan(2006)		Korea(2008)		OECD*	
	US\$**	(%)	US\$**	(%)	US\$**	(%)
In-patient Ser.	987	(38.3)	530	(29.4)	1,663	(55.6)
Out-patient Ser.	823	(31.9)	593	(32.9)	373	(12.5)
Other Ser.	52	(2.0)	10	(0.6)	123	(4.1)
Medical goods	548	(21.3)	463	(25.7)	537	(18.0)
Prevention	60	(2.3)	42	(2.4)	76	(2.5)
Health Admin.	61	(2.3)	56	(3.1)	143	(4.8)
Etc.	49	(1.9)	107	(6.9)	75	(2.5)
Total	2,580	(100)	1,801	(100)	2,991	(100)

1) \* Source: Jeong, Lee, Shin and Song(2008). 17 Countries average including AUS, CAN, USA, ESP, PRT, DNK, NOR, CHE, NLD, KOR, JPN, DEU, FRA, LUX, CZE, SVK and POL.

#### Discussion & Conclusions

The major goal of SHA is to provide a set of international standard aggregating NHA. Estimating THE using common boundary such as OECD/SHA makes it possible to compare the level of health spending, as well as health care system. The SHA manual(ver. 1.0) is scheduled to be revised in several year for further development.

This paper explained about a concept of international classification named OECD/SHA and examined total health expenditure in two Asian countries. Although both of them are based on a same prototype (NHI), it presents the variations in terms of financing sources and allocation of resources. However, the analysis using international data like this is expected to provide some evidence to assess health system performance and to aid policy making.

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Nursing (13:30 ~16:00 Lecture Room 1)

Session chairs: Prof. Makoto Hirai  
Prof. Midori Asano

## Health Status and Health Care Utilization by Marriage-based Women Immigrants in Korea and Policy Directions: Focus on Mental Health

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### Introduction

One of the challenges of the 21st century is providing care to increasingly multicultural societies. Foreigners living in Korea have increased since 1990s. As of December 31, 2009, 2.4% (1,268,477 persons) of the total Korean population were foreigners. Of importance is the rate of increase every year. Korean society is becoming a multicultural society. The rate of international marriage increased in 2005 compared to 2000, but the number of international marriages decreased in 2006 compared to 2005. However it has stood at over 10% of total marriages. Marriage-based women make up 75.5% of couples, which means there are 3 times more foreign women than men in interracial marriages. Most come from China, Vietnam, Philippines. The Ministry of Justice and the Ministry of Gender Equality and Family have a central part in making policies to support marriage-based immigrants. Various services including Korean language, culture, and other services for marriage-based immigrants have been delivered through multi-cultural family centres based on the "Multicultural Families Support Act". However health care policies for these women are relatively scarce. This ever-increasing multicultural population in Korea poses a significant challenge to nurses offering individualized care to their clients. The author reviewed articles and reports related to health status, behaviours, and health care service utilization by marriage-based immigrant women in Korea, and proposed policy directions.

### Conceptual framework and Methods

*Conceptual framework of this research:* 1) National policies and the health care system related to marriage-based immigrants; 2) cultural competence of nurses; 3) cultural adaptation and mental health of marriage-based immigrant women were reviewed based on health determinants of WHO (2005), and behavioural model of Anderson (2008).

*Methods:* Narrative review was done through 3 nationwide reports and 10 research studies published since 2005.

### Results

*National policies and health care system related to marriage-based immigrants:* In 2006 the government instituted a plan consisting of multicultural policies for immigrants to facilitate integration into their communities. First 38 Marriage Immigrant and Family Support Centres were established across the country in 2007, and increased to 171 centres as of 2010. The name was changed to multicultural family support centres according to the "Multicultural Families Support Act [MFSA]" which was enacted on March 21, 2008 by the Ministry of Justice. Maternal and child care are delivered for immigrant women and their children based on MFSA. Examples are provision of helpers after delivery, health screening, and interpreters for health examinations, and medical expenses when a woman delivers a premature baby. These services are supported by the Public Health Centres (PHCs) in 2010.

*Cultural competence of nurses:* There was only one research study on cultural competencies of nurses and care

providers. Cultural competence including cultural behaviour and cultural sensitivity was measured using the Cultural Competence Scale. Scores on cultural behaviour for Public Health Nurses were lower than for care providers at multi-cultural family centres. However scores for cultural sensitivity were not significantly different between the two groups. Public Health Nurses have no educational experience on culture in caring for multi-cultural families.

*Acculturation, mental health status, and health care utilization by marriage-based immigrant women:* Demographic characteristics of marriage-based immigrant women were as follows. The average age of the first marriage for immigrant women was older compared to Korean women (33.3 years of age for immigrant women, 28.7 for Korean women). The average age difference between immigrant women and their partners was 9.9 years, while for Korean couples it was 2.7. In terms of nationality, immigrant women in their twenties at the first marriage came from Vietnam or Cambodia. The average age difference between them and their partners was 17-18 years. Most immigrant women were high school graduates, but 20-30% of women coming from Vietnam or Cambodia had less than elementary school graduation. Regarding length of residence, 90% of women coming from Vietnam or Cambodia had under 5 years and 21.5% of immigrant women's household monthly income was under 1,000,000won (about 73,800 ¥). Medical aid benefits were received by 7.9% of immigrant women, which is higher than the rate for Korean people (3.7%). Regarding their acculturation, the greatest difficulty was language difference. The overall level of acculturation stress was moderate, and cultural shock had the highest score. Taking their health into account, 52.1%-53.8% of the women had a perceived general health status as good which is higher than the 38.7%, rate for Korean women who are over 19 years of age. The prevalence rate for depression was 26.5%-40.6%, also higher than the 18.9%, prevalence rate of depression among Korean women of the same age. The rate of alcohol consumption and smoking was lower than for Korean women of this age. The rate of visits to clinics or hospitals when they felt ill was 73.3-78.6%, while to Public Health Centres was 3.3-8.7%.

### Policy directions

Based on these results, four policy directions are proposed. *A health care system integrated with language, culture, and health literacy is needed:* Language is a body of words and systems of meaning for the use of the words that is common to a people who are of the same cultural tradition or nation. Limited Korean proficiency is to be restricted in the ability to read, speak, write, or understand Korean, so that language difference can cause difficulty in interrelationships among people. The language differences themselves are barriers to effective communication. The inability to communicate with a health care provider not only limits access to health care but also affects the quality of medical care received and appropriateness of follow-up. Culture refers to the learned, shared and transmitted knowledge of values, beliefs and life ways of a particular ethnic group that are generally transmitted inter-generationally and influence thinking, decisions and actions in

patterns or in certain ways (Leininger, 1991). This pattern of beliefs influences how symptoms are recognized and how they are interpreted and affects how and when health services are sought. The level of acculturation results in differences in the use of health care services. One's culture affects one's understanding of a word or sentence and even one's perception of the world. Cultural differences, which are often associated with language differences, are barriers to communication with health care providers. Health literacy is defined as the degree to which individuals have the capacity to obtain, process, and understand basic health information and services needed to make appropriate health care decisions (IOM, 2004). Language and culture provide the experiential context for comprehension of health information. The culturally bound beliefs, values, and preferences a person holds influence how a person interprets health care information. Knowing about a client's language and culture is a key to assessing the health literacy of the client in a given situation. Reducing unnecessary diagnostic testing or inappropriate use of services by providing culturally and linguistically appropriate care through improvements in health literacy leads to efficiency of care. Therefore a health care system integrated with culture and linguistics and giving consideration to health literacy is necessary for quality of care and improvement in the health of immigrant women.

*Development of national standards on culturally and linguistically appropriate services & in-service education on culturally competent health care for public health nurses (PHNs):* Cultural competence is important considering PHNs diverse cultural backgrounds. PHNs often serve as advocates for multicultural families through health promotion interventions and community-based disease control and prevention activities. Being culturally competent allows PHNs to more effectively engage in practice, advocacy activities, and to decrease of health disparities. Nurses are accountable for considering cultural differences when developing care approaches specific to these multicultural individuals and families. Cultural awareness reflects knowledge of the differences among individuals and families. Cultural sensitivity includes one's attitudes towards others and one's openness to knowledge within multiple cultural dimensions. Cultural competence includes actions taken in response to cultural awareness and sensitivity. It involves the ability of individualized care for multicultural women's health. Therefore, local and national public health care systems have to be mandated to provide culturally and linguistically appropriate services through the development of national standards. PHNs and other hospital nurses need to be offered in-service education programs on cultural competency skills. Furthermore, nursing education needs to include trans-cultural nursing in the curriculum. The curriculum in trans-cultural nursing should teach the knowledge and skills needed to provide culturally competent nursing care.

*Development of an assessment instrument for high-risk immigrant women & provision of individualized intervention programs:* The development of an accurate assessment instrument for immigrant women within the community is essential to culturally competent care. An assessment instrument needs to develop to discriminate primarily what is high-risk or not. The instrument including language, culture and health literacy and should be valid and reliable. Based on the literature review, immigrant women's health and health behaviour were found to differ by demographic characteristics, nationality, length of stay in Korea, Korean

language proficiency, and the level of acculturation. Therefore individualized care is needed.

*Vitalizing functions of the Public Health Centres (PHCs):* PHCs provide primary health care. This care is very important for immigrant women. Resolution of the three policies above must precede providing cultural and linguistic competent care with health literacy at PHCs. Health care of marriage-based immigrant women is needed at PHCs including care from the time of entry into Korea, and by life cycle, and according to level of acculturation. PHCs need to play a key role with the network of multi-cultural family centres and other community resources to enhance the health of these women.

#### Conclusions

In an increasingly multilingual, multicultural society, providing high-quality health care requires overcoming barriers such as language, culture, and low health literacy. For the implementation of high-quality health care practices, there is need for redesign in the health care delivery system. The government should also support an integrated health care system that is effective, reliable, and sustainable.

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## Association of Underweight with Low Lymphocyte Count and Prealbumin as Indicator of Malnutrition in Japanese Women

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#### Introduction

Underweight women in their 20s and 30s appear healthy, but may have potential health problems. Previously, we reported that severely underweight Japanese women in their 20s and 30s tended to have decreased lymphocyte counts.<sup>[1]</sup> The lymphocyte count is known to decrease in a poor nutritional state, due especially to protein-energy malnutrition, and it is regarded as a nutritional state indicator.<sup>[2]</sup> Hence, low lymphocyte counts in underweight women might reflect their potential malnutrition. In our previous study, we only examined lymphocyte counts, not albumin or prealbumin. All three are regarded as indicators of malnutrition,<sup>[2]</sup> and are used as popular markers of nutrition screening for hospital inpatients. The aim of the present study was to investigate the health problems of Japanese underweight women in their 20s and 30s who underwent a health check-up by measuring albumin, prealbumin and lymphocyte count as indicators of their nutritional status.

#### Methods

##### Design and sample

A cross-sectional study was conducted with population-based samples. The subjects were recruited from females aged 18-39 years who participated in an annual health check-up from November 6 to 27, 2008, for residents of a city in Aichi prefecture, Japan. Women who were under medical treatment, pregnant or currently breast-feeding were excluded from the present analysis. This left 912 participants (mean age (SD): 33.6 (4.0) years, range 19-39 years) eligible for the present analysis.

##### Measurements

The health check-up included a questionnaire about clinical history and lifestyle, body height and weight measurements, and blood examinations. BMI (kg/m<sup>2</sup>) was calculated as weight in kilograms divided by the square of height in meters. The self-administered questionnaire included questions on smoking, drinking habits, pregnancy, breast-feeding and weight change.

##### Data analysis

BMI was categorized as underweight (BMI <18.5 kg/m<sup>2</sup>), normal weight (18.5 ≤ BMI <25 kg/m<sup>2</sup>) and obese (BMI ≥25 kg/m<sup>2</sup>). Weight change in the last 3 months was categorized as weight loss (of 1 kg and over), no change (within 1 kg) and weight gain (of 1 kg and over). Nutritional assessments were done on the basis of the cut-off values: albumin of <3.5 g/dl, prealbumin of <20 mg/dl and a lymphocyte count of <1500 /μl.

This study was approved by the ethics committee of the Nagoya University School of Medicine.

#### Results

The prevalence of underweight (BMI <18.5 kg/m<sup>2</sup>) was 23.0% (n = 210); normal weight (18.5 ≤ BMI <25 kg/m<sup>2</sup>) was 68.3% (n = 623); and obese (BMI ≥25 kg/m<sup>2</sup>) was 8.7% (n = 79). As for blood examinations, the leukocyte (p < 0.001), neutrophil (p = 0.001), monocyte (p = 0.004), and lymphocyte counts (p < 0.001) declined with a decrease in BMI. Serum prealbumin also decreased with lower BMI (p < 0.001). The prevalence of low lymphocyte counts of less than 1500 /μl was higher in the underweight group (p = 0.001). The prevalence of a low prealbumin level of less than 20 mg/dl tended to be greater among the underweight group, though not significantly (p = 0.098). Serum albumin increased with lower BMI (p = 0.009), but no one displayed a low albumin level of less than 3.5 g/dl. Serum total protein did not differ among the BMI groups.

In a comparison among the weight change groups, BMI tended to be lower in the weight loss group (p < 0.001). Lymphocyte counts (p < 0.001) and serum prealbumin (p < 0.001) were lower in the weight loss group, while serum albumin was higher in the weight loss group (p = 0.012). The prevalence of low prealbumin of less than 20 mg/dl increased with weight loss (p = 0.038), while that of low lymphocyte counts of less than 1500 /μl did not differ among the weight change groups.

The possible association of being underweight and weight loss with a low lymphocyte count of less than 1500/μl or low prealbumin of less than 20 mg/dl was examined by multivariate logistic regression analysis after adjusting for age, smoking and drinking habits. The multivariate logistic regression analysis showed an association of low lymphocyte counts (< 1500/μl) with being underweight (odds ratio (OR) 1.96, 95% confidence interval (CI) 1.35-2.83), but not with weight loss in the last 3 months. On the other hand, low prealbumin (<20 mg/dl) was associated with weight loss (OR 1.42, 95%CI 1.00-2.02), but not with being underweight.

In relation to the BMI and weight loss groups, the prevalence of low lymphocyte counts and that of low prealbumin levels were examined among 647 subjects who had neither smoking nor drinking habits to exclude the influence of the two habits (Table 1). Low lymphocyte counts (<1500 /μl) were most frequently found in 7 (35%) in the underweight plus weight loss groups (n = 20). Further, 4 (50.0%) had low lymphocyte counts (<1500 /μl) among 8 underweight women who lost weight of 2 kg or more in the last 3 months. The prevalence of low lymphocyte counts tended to be higher with lower BMI in the weight loss subjects (p = 0.077) or those without weight change (p = 0.039). On the other hand, the prevalence of low prealbumin (<20 mg/dl) was not at its highest in the underweight plus weight loss groups. The prevalence tended to be higher in the weight loss group in the normal weight (p = 0.010) and obese subjects (p = 0.023).

**Discussion**

The present study showed that underweight women were likely to have lower lymphocyte counts. The prevalence of a low lymphocyte count (<1500 /μl) tended to be higher in the underweight subjects. In addition, the prevalence was higher by 35% among underweight women who lost weight of 1 kg or more in the past three months, and higher by 50% among those who lost weight of over 2 kg. Protein-energy malnutrition (PEM) is known to decrease lymphocyte counts, and lymphocyte counts are used as an indicator of PEM.<sup>[2]</sup> These findings suggested that underweight women in their 20s and 30s can be at risk for low lymphocyte count or potential malnutrition. The risk for malnutrition is thought to be particularly high in underweight women who have lost weight recently.

In our present study, albumin and prealbumin showed no clear association with being underweight. It may be difficult to assess malnutrition by albumin and prealbumin. Earlier studies have reported that concentrations of albumin and prealbumin were within the normal range even in patients with anorexia nervosa.<sup>[3,4]</sup> Other studies have also demonstrated that albumin and prealbumin concentrations did not differ between patients with anorexia nervosa and the controls.<sup>[5]</sup> The present study suggested that lymphocyte counts could serve as a more sensitive indicator of nutritional status in underweight women than either serum prealbumin or albumin. This manuscript has been published in the Journal of Women's Health.<sup>[6]</sup>

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TABLE 1. FREQUENCY (%) OF LOW LYMPHOCYTE COUNT OR LOW PREALBUMIN ACCORDING TO UNDERWEIGHT AND WEIGHT CHANGE GROUPS

	Underweight		Normal weight		Obese		p <sup>a</sup>
	n	%	n	%	n	%	
Weight loss							
Low lymphocyte	7	35.0	7	13.0	1	16.7	0.077
Normal lymphocyte	13	65.0	47	87.0	5	83.3	
No change							
Low lymphocyte	20	17.5	43	14.1	1	2.6	0.039
Normal lymphocyte	94	82.5	261	85.9	38	97.4	
Weight gain							
Low lymphocyte	2	16.7	14	16.5	0	0.0	0.226
Normal lymphocyte	10	83.3	71	83.5	13	100.0	
p <sup>b</sup>	0.129		0.540		0.113		
Weight loss							
Low prealbumin	4	20.0	12	22.2	2	33.3	0.573
Normal prealbumin	16	80.0	42	77.8	4	66.7	
No change							
Low prealbumin	20	17.5	57	18.8	2	5.1	0.262
Normal prealbumin	94	82.5	247	81.3	37	94.9	
Weight gain							
Low prealbumin	2	16.7	6	7.1	0	0.0	0.112
Normal prealbumin	10	83.3	79	92.9	13	100.0	
p <sup>b</sup>	0.790		0.010		0.023		

647 subjects with no habits of smoking and alcohol consumption were used for analysis. Underweight (BMI <18.5 kg/m<sup>2</sup>), Normal (18.5 ≤ BMI <25 kg/m<sup>2</sup>), Obese (BMI ≥25 kg/m<sup>2</sup>). Weight change was classified according to weight changes in the previous three months. Low lymphocyte (<1500 /μl), Normal lymphocyte (≥1500 /μl). Low prealbumin (<20 mg/dl), Normal prealbumin (≥20 mg/dl).

<sup>a</sup> p values by chi-square for trend between indicators of malnutrition with BMI in each weight change group.  
<sup>b</sup> p values by chi-square for trend between indicators of malnutrition with weight changes in each BMI group.

**Comparison of Salivary Cortisol, Heart Rate, and Oxygen Saturation Between Early Skin-to-Skin Contact Methods with Different Initiation and Duration Times in Healthy, Full-Term Infants**

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**Introduction**

Skin-to-skin contact (SSC) is a well-known caring method to facilitate the neurobehavioral self-regulatory responses of the infant after delivery. However, there are few studies that compare the physiological and biological efficacies between different early skin to skin contact (SSC) methods postbirth. The aim of the present study was to investigate how two different SSC methods based on initiation time and duration influenced indicators of stress as measured by salivary cortisol besides time to stability of HR and SpO<sub>2</sub> among healthy full-term infants.

**Materials and Methods**

**Subjects:** We conducted an observational study of 147 consecutive newborn infants who were born spontaneously at two maternity hospitals in Aichi Prefecture, Japan, from January to October in 2009. The subjects were healthy full-term infants who were selected according to the criteria below: Maternal inclusion criteria were spontaneous vaginal delivery, singleton full-term infant, and uncomplicated pregnancy and delivery courses. Maternal exclusion criteria were dysfunctional labor, dystocia, sign of fetal distress during labor, general anesthesia during delivery, multiple birth, and cesarean section. In addition, infants had to be healthy and full term. Infant exclusion criteria were any congenital anomaly, and obvious birth asphyxia as assessed by either 1- or 5-minute' apgar of seven or less. Furthermore, the infants for whom the salivary cortisol levels were obtained at 1min, 60 min, and 120 min were eligible for this study. Finally, the current analysis was restricted to 79 healthy full-term infants, 68 for whom data were obtained on both heart rate and oxygen saturation (figure1). This study protocol was approved by the Ethics Review Committee of the Nagoya University School of Medicine, Nagoya, Japan.

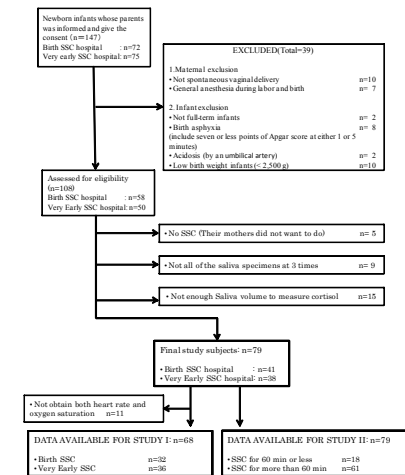
**Salivary Cortisol:** Saliva specimens were collected a total of 3 times; at 1 min after checking 1-min apgar score, and 60 min and 120 min after birth, respectively. Saliva was collected from infants using the Sorbette (Salimetrics, LLC, Pennsylvania, USA). After collection, the saliva was stored at - 83°C. Salivary cortisol concentration was determined using a commercial high sensitivity EIA kit (Salimetrics, LLC, Pennsylvania, USA).

**Heart Rate and Oxygen Saturation:** Pulse oximetry measurements were carried out using a NELLCOR Oxisensor N-25 and a NELLCOR™ OxiMax™ N-600™ Pulse Oximeter (Covidien-Nellcor and Puritan Bennett, Boulder, USA). We placed a pulse oximetry probe over the newborn's right sole as soon as possible after birth. The pulse oximeter recorded arterial SpO<sub>2</sub> and HR every 10 seconds for 120 min after birth.

**Study 1:** To determine whether or not the difference in the initiation time of SSC influences the time to stability of HR and SpO<sub>2</sub> for almost 30 min after birth. The time to stability of HR and SpO<sub>2</sub> were defined as the first of 3 consecutive HR readings of 120-160 beats per minute (bpm) and SpO<sub>2</sub> reading of 96% =, respectively. The first time of 3 consecutive SpO<sub>2</sub> reading of 92% = was also evaluated.

**Study 2:** To assess whether or not the difference in the duration of SSC influences salivary cortisol levels over the first 2 hours after birth.

**Figure 1** Flow of the participants through the study and data collection.



**Data analysis:** In study 1, study infants were divided into two groups: those who began SSC 5 min or less (n=32) after birth and those who did so more than 5 min (n=36) after birth. In the present study, the former group was defined as 'birth SSC' and the latter group as 'Very early SSC' based on the classification of early SSC by Moore et al.<sup>2</sup> Kaplan-Meier curves were generated for time to HR and SpO<sub>2</sub> stability. The difference between groups was assessed with log-rank test. Next, we used Cox proportional hazard model to relate the time of HR or SpO<sub>2</sub> stability to a number of explanatory variables including SSC initiation time category, umbilical artery pH, labor induction, meconium staining, birth weight, and total length of first-stage and second-stage labor in minutes. The exponent of the parameter estimate of SSC initiation time category [EXP(parameter estimate)] indicates the efficacy of Birth SSC group compared with the reference category (Very early SSC group). Additionally, mean HRs were calculated at 1-min intervals from 3 to 30 min after birth.

In Study 2, we divided the subjects into two groups: those who underwent SSC for 60 min or less (n=18) and those who did so for more than 60 min (n=61). As we measured salivary cortisol levels a total of 3 times for 120 min after birth, the

general linear model (GLM) in SPSS that is used in repeated measures designs (two-way repeated measures ANOVA) was used to compare the effect on salivary cortisol levels between the two groups and calculate each mean cortisol level 3 times by 2 groups while adjusting for the initiation time of SSC, umbilical artery pH, labor induction, meconium staining, birth weight, and total length of first-stage and second-stage labor in minutes. Data were analyzed using the SPSS statistical package for Windows Version 17.0 (SPSS, Chicago, USA). Differences were considered significant when  $P < 0.05$  for the two tails.

#### Results

HRs showed a lower tendency among the birth SSC group than the very early SSC group. There were significant differences at 5, 10, 15, and 20 min ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.01$ ,  $P < 0.05$ , respectively). Kaplan-Meier analysis showed a significant difference in the cumulative probability of HR stability of 120-160 bpm between the subgroups ( $P < 0.01$ ). In Cox model analysis, the birth SSC group showed 2.52 times the efficacy of HR stability compared with the very early SSC group [EXP(0.925)=2.52 (95% CI: 1.41-4.51),  $P < 0.02$ ]. Also, the cumulative probability of the SpO<sub>2</sub> stability of 92%  $\leq$  and 96%  $\leq$  using Kaplan-Meier analysis. It did not show any significant difference in cumulative probabilities of either 92%  $\leq$  nor 96%  $\leq$  between the subgroups ( $P = 0.69$  and 0.39, respectively). Cox model analysis also showed no significant associations between initiation time of SSC and SpO<sub>2</sub> stability [SpO<sub>2</sub> stability of 92%  $\leq$  as dependent variable; EXP (parameter estimate)=EXP(0.062)=1.06 (95% confidence interval: 0.609-1.86),  $P = 0.872$  and SpO<sub>2</sub> stability of 96%  $\leq$  as dependent variable; EXP(0.111)=1.12 (95% CI: 0.652-1.92),  $P = 0.652$ ]. Crude means (standard error: SE) of salivary cortisol were 6.10 (0.74)  $\mu\text{g/dl}$  at 1 min, 4.72 (0.50)  $\mu\text{g/dl}$  at 60 min, and 2.71 (0.41)  $\mu\text{g/dl}$  at 120 min in the infants who underwent SSC for 60 min or less, and 6.29 (0.40)  $\mu\text{g/dl}$  at 1 min, 4.04 (0.27)  $\mu\text{g/dl}$  at 60 min, and 2.09 (0.22)  $\mu\text{g/dl}$  at 120 min in those who did so for more than 60 min. The cortisol level for 1 min was not significantly different between the subgroups ( $P = 0.824$ ). Salivary cortisol levels showed decreasing trends in both groups and no significant difference between two groups ( $P = 0.429$ ). After multivariate adjustment, the adjusted means (SE) were 5.03 (0.46)  $\mu\text{g/dl}$  at 60 min and 2.71 (0.40)  $\mu\text{g/dl}$  at 120 min among the infants who underwent SSC for 60 min or less, and 3.94 (0.24)  $\mu\text{g/dl}$  at 60 min, and 2.08 (0.21)  $\mu\text{g/dl}$  at 120 min among those who did so for more than 60 min. SSC continuing for more than 60 min significantly decreased salivary cortisol levels between 60 and 120 min after birth compared with SSC for 60 min or less ( $P < 0.05$ ).

#### Discussion

In our study of healthy full-term infants, we found that birth SSC lead to the stability of HR earlier than very early SSC. All of measured body temperatures postbirth kept in normal range though they were significantly lower in the birth SSC group than the very early SSC group. In addition, SSC continuing for 60= $\leq$  min significantly decreased salivary cortisol levels as a marker for stress at 2 hours postbirth compared with SSC for only  $< 60$  min. The present study proposed further evidences of early SSC for full-term infants.

Most of the studies also compared the infants with SSC with control infants who were separated from their mothers. To our knowledge, there were only a few randomized control studies that compared behavioral and physiological change of infants or mothers between dyads with different initiation

times and/or duration of SSC. In the study by Christensson et al.<sup>1</sup>, 44 healthy full-term infants were observed during the first 90 min after birth in terms of duration of crying when infants were cared with: 1) 76-85 min of SSC with mother. 2) In a cot for the first 45 min of 90 min observation period and then SSC with the mother for 45 min. 3) In a cot for 76-85 min. The cumulative amount of crying during the first 90 min after birth was significantly less in the SSC group than in the mixed cot/SSC group. The findings of this previous study seemed likely to support ours. In the previous studies examining the efficacy of SSC during the early period after birth, infant temperature, HR, respiratory rate, and blood glucose have been used as study outcomes. So we paid due attention to salivary cortisol. We found a significant difference in the effects of SSC on salivary cortisol immediately after birth between infants with SSC of 60 min or less and those with more than 60 min. It is difficult to explain the unitary mechanism underlying the various efficacies of SSC. SSC is suggested to facilitate the neurobehavioral self-regulatory responses of the infants who persist in more labile imbalance and fluctuation of autonomic, motor, state, and attention/interaction subsystem. Our findings are supposed to be due to the postbirth development of self-regulation of each subsystem by SSC. Self-regulation is considered as infant adaptation to various internal and external stimuli and to unstable situations, and is in great demand immediately after delivery. Early SSC may be a 'nature way' of antagonizing the 'stress of being bone' like the other mammals. Therefore, 'When should SSC start after birth?' and 'How long should SSC be underwent?' are the important problems in establishing the effective SSC. Additionally, it is also necessary to establish the safety SSC method.

There are two limitations in our study. One limitation was that our study was observational. Thus, subjects must be randomized to confirm our results even enough, though the characteristics of SSC groups were almost comparable and multivariate adjustment was performed. Another limitation arises from the fact that healthy and full-term infants were the subjects of our study. Hence, our results may not apply to preterm infants and infants with any complication.

#### Conclusions

Birth SSC lead to the stability of HR earlier than very early SSC. All of measured body temperatures postbirth kept in normal range though they were significantly lower in the birth SSC group than the very early SSC group. In addition, SSC continuing for 60= $\leq$  min significantly decreased salivary cortisol levels as a marker for stress at 2 hours postbirth compared with SSC for only  $< 60$  min.

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## Evidence-Based Nursing Strategy for Acute Ischemic Stroke Patients Management in the Emergency Department

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#### Introduction

Stroke, a sudden loss of neurologic function resulting from an acute disruption of blood flow, is the leading cause of long-term disability and the second leading cause of death in South Korea.

Emergency nurses are often the first health professionals to come into contact with stroke patients on arrival at hospital, but their understanding of the basic management of acute stroke is often limited[1]. An important part of acute stroke management and decreasing stroke-related mortality is preventing complications within the first 24-48 hours[2].

The purpose of this study was to establish an evidence-based nursing strategy for Emergency Department nurses to aid in understanding basic management and preventing of complications of acute ischemic stroke.

#### Method

This study was performed using a methodological design. Referring to four Stroke Guidelines by the Clinical Research Center For Stroke in South Korea. And the Detailed Nursing Strategy was obtained through the procedure of critical literature analysis.

#### Nursing Strategies: ABCDs of Acute Stroke Management

##### A IS FOR AIRWAYS

Obstruction or dysfunction of the airway is not uncommon in acute stroke. To ensure that the patient's airway is maintained during acute ischemic stroke, nurses should be prepared to suction, control the airway, and prevent aspiration. Patients unable to handle secretions need to be intubated to protect the airway. Initially, if the level of consciousness is decreased, but the ability to swallow is intact, the patient may only need an oral or nasal aid to maintain the airway.

Emergency department nurses are asked frequently to give medications or fluids to a stroke patient. Dysphagia is a sign of a potentially dysfunctional airway and an obvious risk for aspiration. In one study of 30 patients with acute stroke, dysphagia was noted in 57%. These patients had longer hospital stays, more complications, and a higher rate of morbidity[3]. Assessment of swallowing ability should be carried out according to institutional procedure guidelines. Following a dysphagia screening protocol, or algorithm, is recommended as standard practice in the evaluation of patients with acute stroke[4]. Should the patient choke, gag, or gasp for breath before or during screening, he or she should be kept nothing by mouth (NPO). Brain stem infarcts increase the risk that the patient will have speech and swallowing difficulty. Patients passing the swallowing screen can start nutrition early, but reassessment of swallowing is recommended because symptoms may worsen or fluctuate with time.

##### B IS FOR BREATHING

The penumbra consists of potentially salvageable brain cells, so it is critical that oxygen-saturated blood is provided to that area early during a stroke. As little as 2 L/min of supplemental nasal oxygen can raise oxygen saturation from 90% to more than 95%, which in turn may improve tissue oxygenation[5]. If breathing effort is inadequate the patient will require ventilatory assistance to supply sufficient oxygen to the brain. If a patient is experiencing a rapid deterioration secondary to increased intracranial pressure it may be useful to hyperventilate the patient to lower the partial pressure of carbon dioxide (pCO<sub>2</sub>). Carbon dioxide is a potent vasodilator and lowering its concentration may aid in constriction of the vascular compartment and rapidly reduce intracranial pressure. However, excessive lowering of pCO<sub>2</sub> can lead to increased ischemia. The goal therefore is to lower pCO<sub>2</sub> to 25 mm Hg.

Breathing rate and rhythm is controlled within the brainstem and can be affected by brain swelling. Downward displacement of the brain and brain stem can be caused by edema, increased blood flow, or blood from a hemorrhage. This can lead to ventilatory pattern changes as the body tries to decrease the size of the vascular bed by blowing off carbon dioxide. Abnormal ventilatory patterns and yawning are signs of increasing pressure in the brain and on the vagus nerve(that controls respiratory muscles) as the nerve is squeezed through the foramen magnum(herniation). A stroke occurring within the arterial blood flow of the brainstem or cerebellum can also lead to respiratory pattern changes. In addition to oxygen saturation, patients should be monitored for respiratory rate and rhythm.

##### C IS FOR CIRCULATION

The irreversible neurologic deficits of a stroke result when blood flow to an area of the brain is reduced to approximately 25% of the normal value[6]. This loss of perfusion leads to a lack of oxygen and glucose to the cells, resulting in cell death[7]. Cells in the surrounding penumbra may still be viable[8]. Because blood flow in the penumbra is also decreased, perfusion to this ischemic area needs to be reestablished quickly. Action taken during this critical early phase of a stroke can improve or maintain circulation, which allows sufficient oxygen and glucose to reach the neurons in this vulnerable area.

In acute stroke, circulation can be hindered by the increasing mass(eg, from edema) within the skull[9]. A normal response to the lack of oxygen within the brain is a rise in blood pressure. Although rising blood pressure can increase perfusion to the brain for a short time, it may lead to edema, increased intracranial pressure, and an increase in the size of brain mass and potential herniation[10].

Rapidly lowering blood pressure in the ischemic stroke patient can lead to decreased perfusion of the already stressed brain cells. Nurses involved in stroke care need to be aware that cerebral perfusion pressure(the difference between mean arterial pressure and intracranial pressure) should be maintained at a level above 40 mm Hg to obtain adequate blood flow to brain tissue. Systolic, diastolic, and mean arterial blood pressure (MAP) will be higher than usual, as MAP below 100 mm Hg have been shown to correlate with poorer outcomes[11]. The



exception is in patients who receive rt-PA: blood pressure will need to be lowered to prevent complications from the medication during reperfusion.

Classically, in patients with impairment of cerebral circulation due to a clot, reperfusion is attempted using fibrinolytic therapy (eg, rt-PA). Nurses should know the inclusion criteria to identify patients for such therapy, as rapid evaluation for appropriateness and initiation of administration of fibrinolytic therapy is critical in preventing cell death [12]. It is important to remember that there is only a 3-hour window from onset of symptoms in which intravenous rt-PA can be given, after which the risk of bleeding increases. Nurses should also facilitate obtaining appropriate lab tests and a brain CT scan, with the highest priority placed on rapidly securing the scan. The CT scan must be completed and results obtained before a patient with suspected ischemic stroke can be treated with fibrinolytic therapy. Time is critical, and all members of the stroke care team need to expedite the process. Communication among pre-hospital personnel, ED nurses and doctors, and radiology staff can produce a door-to-CT time of just a few minutes.

#### D IS FOR DISABILITY AND DEXTROSE

Because stroke is a leading cause of long-term disability, nurses should be able to quickly identify the patient with stroke symptoms. Assessment tools that can quickly identify the patient at risk for stroke are the Face Arm Speech Test (FAST) and the Los Angeles Prehospital Stroke Scale (LAPSS). Either can be administered by the ED nurse in triage or by paramedics before reaching the emergency department. Patient information from these assessment tools can be re-verified by the ED nurse, physician, or critical care nurse using the more comprehensive National Institutes of Health Stroke Scale (NIHSS) [13]. The NIHSS provides a standardized baseline and consistent set of parameters that can be repeated throughout the hospital stay. Some stroke centers order a CT scan based on results of the FAST or LAPSS and then carry out the NIHSS assessment on the patient's return to the ED after imaging is complete.

Many have seen how the neurologic effects of low serum glucose (dextrose) levels can mimic the symptoms of acute stroke. A finger-stick glucose test should be done early to rule out hypoglycemia. In fact, if the glucose level was done pre-hospital and results were marginal, a repeat finger-stick test might be in order.

In the acute stroke patient, hyperglycemia is a predictor of poor outcome. Stress of a stroke has been seen to increase blood sugar even in the nondiabetic. Hyperglycemia is associated with longer hospital stays (7.2 days vs. 6 days), increased risk of bleeding into the penumbra, and an increased risk for death at 30 days [14]. The stroke patients who have their blood sugar tightly controlled early on with hourly blood glucose checks and intravenous insulin, seem to have less bleeding into the penumbra, and better outcomes.

#### Conclusions

Public awareness of stroke symptoms as well as rapid and effective assessment of suspected stroke is critical for optimum patient outcomes.

Rapid and accurate assessment of patients with suspected stroke by emergency nursing personnel is essential. The early stages of patient stabilization and diagnosis are critical to brain function; thus, it is imperative to assess, monitor, and correct the ABCDs during emergency stroke management. These initial steps are not only crucial for maintaining brain function, but are invaluable for salvaging penumbral cells. Moreover, the prompt recognition of stroke will lead to

quicker implementation of treatment and to effectively treating this disease as a high-priority emergency. Additionally, continued monitoring of the ABCDs can lead to early recognition of patient deterioration. Rapid intervention and preventing poststroke complications are vital when emergency nursing personnel evaluate stroke.

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## DEVELOPMENT of a JAPANESE FUNCTIONAL HEALTH LITERACY TEST

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#### Introduction:

The importance of health literacy has been highlighted in relation to health prevention and the health promotion. "Health literacy is a topic that has been gaining momentum over the past few years, in the policy research agendas of many countries" [1]. Thus a major goal of the World Health Organization, Healthy People 2010, and the Joint Commission is to improve health communication quality and provide safe healthcare for patients with low health literacy [2-4].

Nutbeam [5] suggested that functional health literacy was one of the subordinate concepts of health literacy, in which there were two other conceptual configurations, interactive health literacy and critical health literacy. According to Nutbeam [5], Speros [6] and Mancuso [7], health literacy is cognitive and social skills, ability of individuals to gain access to, understand, and use health information that promote and maintain good health. Thus, functional health literacy is that person's ability to numerate, read, and comprehend basic medical terms required to function in the health care environment. Furthermore, it takes various forms such as: basic numeracy, reading and comprehension skill that allow a person to function in the health care environment; the ability to apply literacy skills to material such as prescriptions, appointment cards, medicine instructions, and directions for self-management of diseases and conditions; the abilities to understand and act on information and instructions from professionals, for example informed consent form or brochures; and patient information-seeking and shared decision-making with health professionals.

In the United States and other developed countries, evidence shows that patients with inadequate health literacy have lower adherence to medical instructions [8], lower use of preventive services [9, 10], poor understanding of one's medical condition, being into a difficult situation to seek the medical information by oneself [11], higher hospitalization [11, 12], and increased mortality [13]. In recent studies, between 40 and 50% of U.S. adults with lack of the reading and numeracy skills to fully understand the health information are substantial [14]. Kim indicated that 35% of Korean adults had low functional health literacy level, below Korean elementary school level [15]. Tokuda showed that about 36% of Japanese people had poor health knowledge [16]. Japanese literacy rates are very high, as most people are able to read easy texts as well as newspaper, but we have to keep in mind that most Japanese patients can understand what medical staff said and showed instruction papers. Now, there are likely to be certain evidence about the relationship between the inadequate health literacy and health outcomes.

This evidence has been shown by two English health literacy tests, such as Rapid Estimate of Adult Literacy in Medicine (REALM) [17] and The Test of Functional Health Literacy in Adults (TOFHLA) [18], and one Japanese health knowledge test, Japanese Health Knowledge Test [16]. REALM was developed in 1995, and has been used most frequently. This test includes only assessing pronunciation of the medical terms correctly. This has been utilized by only

English speakers. TOFHLA was developed to measure the participants' ability to successfully complete basic numeracy and reading health literacy skills in 2001. This test includes one numeracy and two reading passages to assess comprehension of labeled prescription vials and hospital forms. This has been also utilized by only English speakers, because these questions are based on the American health care system. Thus, it is invalid to appraise the Japanese functional health literacy. Japanese health knowledge test was developed to estimate health knowledge among general public, and in identifying and characterizing Japanese with poor health knowledge, in 2010. This test is only able to measure knowledge of 15 health related vocabularies for ordinary people. In a health care setting, it is required to measure patient's abilities that focus on not only knowledge but skills relating primarily to health care settings. Therefore, there has been no scale or test to measure ability of Japanese functional health literacy yet. We will develop and examine functional health literacy test for Japanese adults.

#### Methods:

##### Study participants

The data for study will be collected from about three hundred fifty out patients, whose first language is Japanese, are age 22 years or over, can answer very easy questions such as where he/she lives at then and what day is then, are not blind and deaf. Patients younger than 22 years old will be excluded, because one of the aims of this study includes evaluating the potential relationship between the functional health literacy and the final educational attainment. Medical service personnel, such as physicians, nurses, pharmacists, hospital workers, and caregivers, will be excluded.

##### Development of the Japanese Functional Health Literacy Test (J-FHLT)

This study is designed for a survey to examine the construct validity and the item adequacy. First item pool of 72 was generated based on REALM, TOFHLA, J-HKT, and empirical referents, such as Japanese medical system, health education or information materials from physicians or nurses, prescriptions or materials for medicine, and information documents of X-ray examination or endoscopic examination.

The first item pool was examined by an advisory panel of nine nursing students in the first field test, and it was refined and reduced items to 34 items.

The second item pool consisted of three scales: eight numeracy items, fifteen reading items, and eleven comprehension items. Content validity for this second item pool was tested by ten content experts panel, such as two doctors, two medical researchers, two nurses, two pharmacists, two educational researchers. These experts were given a rating form with functional health literacy definition and items. They were required to review each item for validity, difficulty, and readability with a 4-point scale (from 1 = no valid to 4 = very valid). At the present stage, we calculate the index of content validity (CVI). CVI is classified according to Lynn's criteria [19] that are related as over 3 by the experts and the median over 3.

#### Data collection

This study also collects demographic and socioeconomic data. Demographic data is comprised of age, sex, annual income, final educational attainment, and occupation.

Furthermore, we measure three questions, translated into Japanese, from the Health Literacy Screening Questions (HLSQ) [20] and J-HKT for calculating criterion-related validity. Three questions of the HLSQ, "how often do you have someone help you read hospital materials?", "how confident are you filling out medical forms by yourself?", and "how often do you have problems learning about your medical condition because of difficulty understanding written information?" were each effective 5-point Likert screening tests for inadequate health literacy in the U.S. patients.

#### Planning schedule: Fig. 1

First of all, we are trying to refine the second item pool based on CVI and the median.

Second, we will conduct the second field test with about fifteen out-patients and refining the item pool again.

Third, the second item pool based on the field test will be generated.

Fourth, the second item pool will be tested with about three hundred fifty out-patients as third field test.

Fifth, we will go over factor analysis and calculate internal consistency reliability (Cronbach's alpha) and criterion-related validity (validity coefficient) from third field test data. We also use the Rasch analysis to evaluate the adequacy of items. Taking advantage of Rasch analysis, we calculate item difficulty, item-fit indices, and person separation reliability in order to exhibit the degree to which individual items clarify the unidimensionality of the construct "health literacy."

Sixth, for the purpose of taking a final check, we will recalculate internal consistency reliability (Cronbach's alpha) and criterion-related validity (validity coefficient) by use of data from third field test.

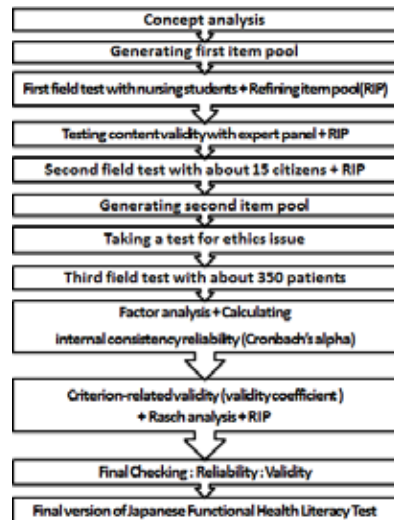


Fig. 1 schedule for the study.

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## Related Factors of Self-Efficacy in Cancer Outpatients Receiving Chemotherapy in the National University Hospital

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#### Introduction

After the medical fee for outpatient cancer chemotherapy was accepted in 2002, the place of chemotherapy has been shifting from hospitalization to outpatient care. Owing to the outpatient chemotherapy, patients can spend daily life receiving chemotherapy, and it contributes to improving their QOL. On the other hand, good self-caring behavior is essential to improve the QOL, and to display good self-caring behavior, patients need to possess high self-efficacy.

#### Purpose

This study aimed to identify by a survey questionnaire the factors related to self-efficacy in cancer outpatients receiving chemotherapy, and to discuss the appropriate nursing care for them.

#### Methods

**Subjects:** The subjects consisted of 169 cancer outpatients receiving chemotherapy at the national university hospital's chemotherapy center from August to September in 2009. The study was approved by the Nagoya University's ethical committee and hospital's ethical committee, and informed consent was obtained from each patient.

**Questionnaire:** The questionnaire covered patient backgrounds, their needs in terms of home care, a Screening Questionnaire (physical symptoms, the Severity Distress Thermometer, overall quality of life) and the Self-Efficacy Scale for Advanced Cancer (SEAC). SEAC consisted of three subscales; Affect Regulation Efficacy (ARE), Symptom Coping Efficacy (SCE) and ADL Efficacy (ADE).

**Data analysis:** The relation between self-efficacy and patient backgrounds and their needs was analyzed using the following statistical analysis: Mann-Whitney's U test, Kruskal-Wallis test with Bonferroni correction for multiple comparisons. And relation between self-efficacy and physical symptoms, QOL psychological distress was analyzed using the following statistical analysis: Spearman rank correlation coefficient, multiple linear regression analysis. PASW version 17.0 for Windows was applied for data analysis. Significance was set at  $P < 0.05$ .

#### Results and discussion

The average age of the participants was 59.2 years old, and 43.2% of the participants were males, 56.8% were females. On Performance Status, PS0 was 34.3%, PS1 was 56.8%. On primary site, 30.2% of participants had breast cancer, 23.1% had gallbladder or pancreatic cancer, 18.9% had colorectal cancer.

**The relation between patient backgrounds and self-efficacy:** The score of SEAC was significantly lower among patients with the following backgrounds: under 40 years old, jobless, solitary life, PS1 or more, no hobby, no adviser, and difficulty in consulting doctors or nurses. In detail, the score of ARE and SCE were significantly lower among the patients under 40 years old, and the score of ADE was significantly

lower among jobless patients and patients having solitary life. The score of all three subscales was significantly lower among the patients who do not have hobby, the outpatients receiving chemotherapy for 1-2 years and the patients whose PS is more than 1. And the score of ARE and ADE were significantly lower among the patients with no adviser or difficulty in consulting doctors or nurses. The score of SCE was significantly lower, only among the patients with difficulty in consulting nurses. These findings suggest that nurses take an important role in the symptom management for the patient. They also suggested that we must support the outpatients in consideration of their individual backgrounds and provide the environment where outpatients are easy to consult.

**Relation between their needs in terms of home care and self-efficacy:** The score of all three subscales was significantly lower among the patients having troubles with adverse effect management. In detail, the score of SCE and ARE were significantly lower among the patients who cannot cope with the aggravation of general condition due to severe symptom and those who cannot go out alone. And the score of ARE was significantly lower among the patients who cannot eat what they want. And also, the score of all three subscales was significantly lower among the patients who do not have an adviser and those who cannot express their feeling well. The score of ADE was significantly lower among the patients who feel anxious about a relationship with the doctor. As a result, these findings suggest the necessity to support the patients having these factors and their family in early stage.

**Relation between physical symptom and self-efficacy:** The severity of the following physical symptoms significantly affected the SEAC score: insomnia, inappetence, dyspnea, and fatigue. In detail, the score of ARE was significantly affected by insomnia and fatigue. The score of SCE was significantly affected by appetite loss and dyspnea. The score of ADE was significantly affected by appetite loss and insomnia. As a result, these findings suggest that the nursing system to improve the symptom management and self-caring behavior should be established.

**Relation between QOL/ Psychological distress and self-efficacy:** The scores of the distress thermometer were significantly affected by ARE and ADE, and the scores of QOL were also significantly affected by ADE and ARE. In addition, a significant negative correlation was seen between the distress thermometer and QOL. The nursing care which enhances self-efficacy comprehensively can be the essential for the cancer outpatients (Figure 1).

#### Conclusions

We identified the following factors of self-efficacy in cancer outpatients receiving chemotherapy: patient backgrounds, physical symptoms, patient's needs in terms of home care.

For improving their self-efficacy, it is significant to understand patient's background and their needs sufficiently and to support them individually. And we must make an effort to

palliate the following symptoms especially: insomnia, fatigue, appetite loss, dyspnea.

To generalize these findings, it is necessary to investigate a longitudinal study in the future.

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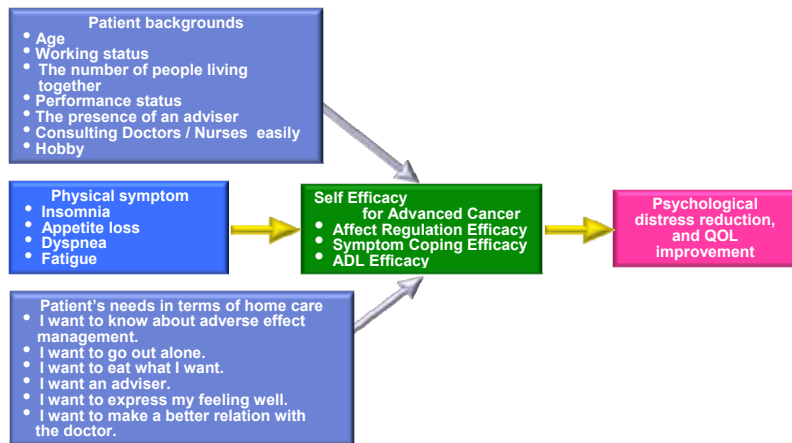


Figure 1 Related Factors of Self-Efficacy in Cancer Outpatients Receiving Chemotherapy

Radiological Technology (13:30 ~16:10 Lecture Room 2)

Session chairs: Prof. Yoshie Kodera  
Associate Prof. Masatoshi Tsuzaka

## ADVANCEMENT OF MEDICAL IMAGING AND INSTRUMENTATION IN RADIOLOGICAL SCIENCE

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### Background

Clinical applications as well as research activities in medical imaging and instrumentation are rapidly growing fields in radiological science. Recent trends of clinical research of SPECT/CT, PET/CT, and PET/MRI as well as advanced imaging modalities indicate that clinical applications of various imaging modalities are very active, and have been triggering research activities of medical imaging and instrumentation in radiological science. Dept. of radiological science at Yonsei University is relatively new and has been focusing on both research of new technologies in medical imaging and instrumentation, and education and training of practical as well as advanced technologies in radiological science.

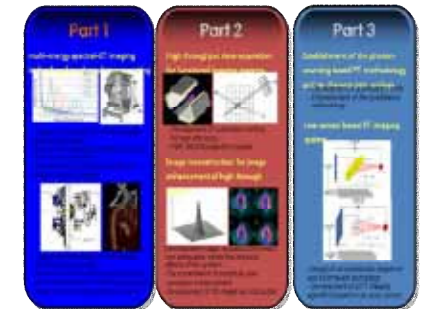
### Methods

Recently, two international congress meetings were held and one international conference meeting will be held in 2013 in Seoul. One was the World Congress on Medical Physics and Biomedical Engineering 2006 with the theme of "Imaging the future medicine" and the other one was "9th Congress of the World Federation of Nuclear Medicine & Biology with the theme of "Global harmonization and new horizon of nuclear medicine" at the same place of Coex, Seoul, Korea. These two congress meetings may cover worldwide major research activities in medical imaging and instrumentation and played a very important role to have nationwide attention including governmental level in Korea. Recently, it's been decided to have 2013 IEEE NSS/MIC conference meeting in Seoul, Korea, and our Dept. will play a major role to host this prestigious conference meeting for the 1st time in Asia and Oceania. There are 4 Laboratories in our Dept. including medical physics and imaging Lab (MPIL), iTOMO Lab, Molecular Imaging Lab (MoIL), and magnetic resonance imaging Lab (MRIL). Major interests of iTOMO Lab includes dental panorama imaging technique, dental CT technique, dual-energy radiography imaging technique, tomosynthesis imaging algorithm, design digital processing filter and algorithm to improve image quality, and image quality estimate technique of digital radiography. MRIL has been researching on functional MRI, diffusion tensor imaging & fiber tractography, and phase contrast MR angiography. MoIL has been researching on developing both small gamma camera and small PET, and image optimization. Research interests of MPIL includes the measurements of physical characteristics of SPECT/PET/X-ray & CT, and their quantitation, imaging physics and instrumentation, and simulation of multi-modality imaging techniques. Recently, our Dept. awarded government grant of "Basic Atomic Energy Research Institute (BAERI)" which will support for next 6 years to develop functional and anatomical multi-imaging technology based on multi-energy photon counting. The purpose of this project is to develop the image acquisition technique for the optimization of multi-energy photon counting images included in functional/anatomical information and to

research the functional and anatomical fused imaging on the state-of-the-art technologies.

### Results

With these activities, in recent years, medical imaging and instrumentation, such as digital x-ray radiography, computed tomography, nuclear magnetic resonance, emission tomography, animal imaging devices, multi-modality systems, and modeling and evaluation of imaging systems, have been rapidly advancing in Korea. The Korean government has been trying to lay a foundation for medical devices and information by providing policies and systematic funding supports on the product and technology development. Based on the government's supports, there are strenuous and growing efforts to improve the nation's international competence made in research and development of medical imaging technology and its applications by universities, industries, and national science research institutes, although major research activities have been



leading by academic side compared to industry side. The figure1 shows the scope of the BAERI project to be developed.

Figure 1. The scope of the development of multi-imaging technology based on photon counting

### Conclusion

Summaries of the current achievements and the future prospects in the realm of medical imaging and instrumentation in our Dept. will be presented with overview of clinical applications in advanced imaging technologies. Summaries of educational system in radiological science as well as medical physics in our Dept. will also be presented.

## NONINVASIVE ESTIMATION OF REGIONAL CEREBRAL BLOOD FLOW USING <sup>123</sup>I-IMP ACQUISITION DATA

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### Introduction

N-isopropyl-p [I-123] iodoamphetamine (<sup>123</sup>I-IMP) is widely used as a tracer for the measurement of regional cerebral blood flow (rCBF) by single-photon emission computed tomography (SPECT). <sup>123</sup>I-IMP is one of the most suitable available SPECT tracers because the radiopharmaceuticals excel in the contrast between the cerebral normal and defective parts. Measurement of rCBF is necessary for evaluation of the cerebral circulation to understand abnormalities of blood flow and the target changes due to disease processes. In the quantification, <sup>123</sup>I-IMP has a higher first-pass extraction fraction and negligible back diffusion from cerebral tissue immediately after injection. Therefore, it is not easily influenced by metabolic activity of the tissues and indicates the good linearity of the radioactivity distribution in rCBF.

In general, arterial blood sampling and an integral of input function are needed to calculate rCBF. SPECT and continuous arterial blood sampling on the basis of the microsphere model described by Kurl et al. were used to measure rCBF with <sup>123</sup>I-IMP (1-2). Moreover, several studies have been made using a two-compartment model analysis which correlates with back diffusion of <sup>123</sup>I-IMP (3). These methods require arterial blood sampling which is invasive for patients and demanding in a daily clinical setting.

To date, there have been some reports on the estimation of rCBF by observing pharmacokinetics of <sup>123</sup>I-IMP without arterial blood sampling (4-5). However, the rCBF values estimated in these studies have not been compared with those obtained by other methods. <sup>15</sup>O-water seems to be more suitable for rCBF quantification than <sup>123</sup>I-IMP because it is not metabolized inside of the body and the extraction fraction is comparatively high (0.85-0.95) (6).

The purpose of this study was to evaluate a new noninvasive method using <sup>123</sup>I-IMP for estimation of rCBF without any blood sampling and to compare the new method with the conventional method using <sup>15</sup>O-water PET.

### Materials and Methods

Consecutive 8 patients with suspected CBF disorders (age: 58±19y, male/female:4/4) underwent PET and SPECT examinations on the same day. Procedures for data acquisition by PET and gamma camera are summarized in Fig.1.

The study was performed according to the approval of the Ethics Committee of Medicine at Nagoya University for Human Studies, and informed consent was obtained from all patients before the study.

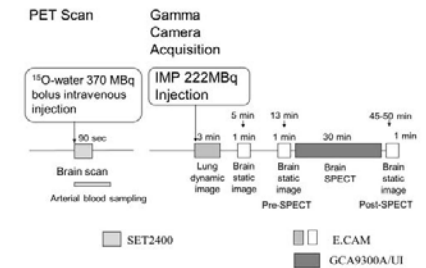


Fig.1 Outlines of the procedure of <sup>15</sup>O-water PET and <sup>123</sup>I-IMP data acquisition with gamma camera.

CBF measurement by PET was performed using <sup>15</sup>O bolus administration autoradiography method by Kanno et al. (6). PET scanner SET2400 (Shimadzu Corporation, Kyoto, Japan) and two gamma cameras E.CAM for two-detector type and GCA9300A/UI for three-detector type (Toshiba Medical systems company respectively, Nasu, Japan) were used. First, CBF measurement by PET was performed. Next, dynamic lung and static head images were acquired after injection of <sup>123</sup>I-IMP, followed by brain SPECT. Lung washout counts and the time course of brain counts were used for explanatory variables, and rCBF measured by <sup>15</sup>O-water PET is used for the dependent variables. The regression equation was calculated with both variables. The rCBF was estimated using the regression equation.

The PET and SPECT images were matched to a standard cerebral template by analytical three dimensions ROI software (7), and the accumulation ratios were calculated on each region of the brain.

### Results

Examples of lung and brain images and lung washout curve for a study patient are shown in Fig. 2. Fig. 3 shows comparison of the rCBF values estimated by <sup>123</sup>I-IMP SPECT and those actually measured by <sup>15</sup>O-water PET autoradiography. A significant correlation ( $r=0.93$ ,  $p<0.001$ ) was detected between rCBF values measured by <sup>15</sup>O-water PET and those estimated with <sup>123</sup>I-IMP.

# AN INFLUENCE OF THE BEAM QUALITY ON THE NOISE OF THE IMAGE IN DIGITAL MAMMOGRAPHY

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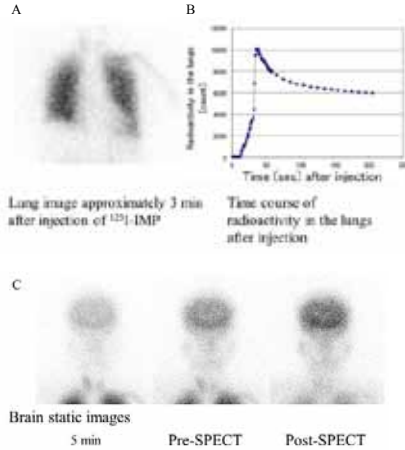


Fig.2 Data acquired after intravenous injection of <sup>125</sup>I-IMP. (A) Lung planar image approximately 3 min after injection. (B) Time course of radioactivity of the lungs after injection. (C) Brain static images at 5 min, 13 min (pre-SPECT) and 45-50 min (post-SPECT) after injection.

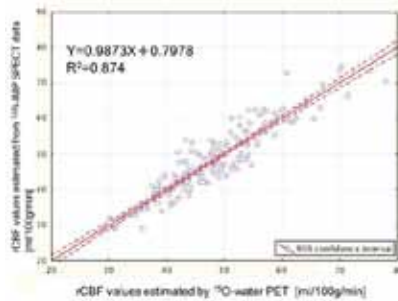


Fig.3 Comparison of the rCBF values estimated by <sup>125</sup>I-IMP SPECT and those measured by <sup>15</sup>O-water PET. There is a significant correlation between the regional CBF values obtained by these two methods ( $r=0.94$ ;  $p<0.001$ ).

## Discussion

We demonstrated that rCBF can be estimated by using the <sup>125</sup>I-IMP initial circulation images of the lungs and brain without any blood sampling, and that the rCBF values obtained by this method showed accuracy similar to those actually measured by <sup>15</sup>O-water PET.

Furthermore, the new method proposed in this study is more useful for daily clinical rCBF quantification than the conventional methods which require arterial blood sampling.

## Conclusion

The noninvasive method proposed in this study can reliably estimate rCBF using brain <sup>125</sup>I-IMP SPECT data without any blood sampling.

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## Introduction

The uses of digital mammography systems have become widespread recently. However, the optimal exposure parameters are uncertain in clinical. We need to optimize the exposure parameter in digital mammography with maximization of the image quality and minimization of patient dose. The purpose of this study is to investigate an influence of the x-ray energy on the noise of the image. We obtained images for various tube voltage and the thickness of compressed breast. The noise properties of these images were evaluated with these indices: Wiener spectrum (WS), the noise equivalent number of quanta (NEQ), and signal-to-noise ratios derived from the perceived statistical decision theory model with the internal noise of eye-brain system (SNR<sub>i</sub>). We measure them under the fixed average glandular dose (ADG).

## Materials and Methods

**Equipment used in this study** ; The mammography equipment used in this study was Mermaid model MGU-100B. The computed radiography (CR) reader used was REGIUS V stage, Model 190. The CR plate used was CP1M200 (with the columnar crystal phosphors). These equipments were manufactured by Konica Minolta MG. The x-ray images of low contrast objects were obtained for the measurement of the SNR<sub>i</sub>. To measure the contrasts and to calculate Wiener spectrum, we obtained images of acrylic steps (thickness: 1-10 mm) and uniformly exposed x-ray images. We used acrylic plates (thickness: 2, 4, 5 and 7 cm) as breast phantoms. Mo/Mo and Mo/Rh were chosen for the target/filter combinations. As the source of low contrast signal, we used a resinous disc (diameter: 4.2mm, thickness: 4.5mm). To measure the presampled modulation transfer function (MTF) for NEQ, we obtained images of the tungstenium edge.

**Measurement of the average glandular dose** ; We measured the average glandular dose (AGD) and chose the exposure level which gave the European Reference Organization for Quality Assured Breast Screening (EUREF) dose acceptable level for every combination of the thicknesses of phantoms and the tube voltages (25, 28, 30, 32, 35 kV). Actually, we could not adjust the exposure level for the AGD strictly because of the restriction of the machine. Therefore we selected the exposure level for EUREF dose acceptable level as near as possible (Table.1 and Table.2).

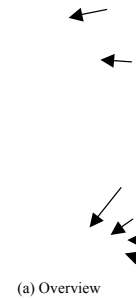
**Measurement of image contrasts** ; WS depends on the image contrast, and the image contrast changes with the tube voltage. For this reason, we evaluated WS under fixed image contrast. In order to do this, the image contrast of each tube voltage was measured. We obtained the images of the acrylic plates and the acrylic steps, which were put on the chest wall side of the table for the contrast evaluation (Fig. 1). We read out the raw data of the acrylic step images and found the contrast by the pixel value of each step.

Table 1. EUREF dose acceptable level for each thickness of PMMA and its equivalent breast thickness

PMMA thickness [cm]	Equivalent breast thickness [cm]	Dose acceptable level [mGy]
2	2.1	0.6
4	4.5	1.6
5	6.0	2.4
7	9.0	5.1

Table 2. Exposure level for 4-cm-thick PMMA with Mo/Mo

AGD [mGy]	25 kV	28 kV	30 kV	32 kV
1.3	80	50	40	32
1.7	100	63	50	40



(a) Overview



(b) Overhead view

Fig. 1 Layout of measurement of the contrast

**Calculation of WS** ; We calculated the WS by the two-dimensional fast Fourier transform method. When we evaluated the WS values at 25, 30, 32 and 35 kV, we adjusted the contrasts under these tube voltages to that under 28 kV. To tune the image contrasts, we computed the WS and multiplied the following correction factor  $k$ .

$$k = (\text{image contrast at 28 kV} / \text{image contrast at another tube voltage})^2$$

**Calculation of NEQ** ; The NEQ is described as shown below:

$$NEQ = \{(G \cdot \log_{10} e)^2 MTF^2\} / \{WS_{APV}\}$$

In these figure,  $G$  means the gradient of the characteristic curve for the detector.

**Calculation of SNR<sub>i</sub>** ; The SNR<sub>i</sub>, which takes the spatial frequency response of the human visual system and the internal noise of eye-brain system into consideration, is written as

$$SNR_i = SNR_p / \{1 + (N_i / N_p)^2\}^{1/2}$$

In this formula, the SNR<sub>p</sub> represents the ratio of signal ( $S_p$ ) to noise ( $N_p$ ). The value of the  $S_p$  and the  $N_p$  were calculated in consideration of the visual spatial frequency response of the human observer<sup>3)</sup>. The  $N_i$  means the internal noise<sup>4)</sup> which is inherent in an observer, for instance, the noise in relation to the neurophysiological unstableness and to fluctuations of the observer's memory.

#### Results

**WS** ; The WS was almost stable when the tube voltage changed between 2-cm and 4-cm-thick phantoms. There were few differences in the WS values for each tube voltage with 5-cm-thick phantom, and we observed the tendency that high tube voltage improves the noise property of the image. This trend was more remarkable for 7-cm-thick phantom. As the combination of target/filter, Mo/Rh was more beneficial than Mo/Mo for phantoms thicker than 4 cm. This trend was also more conspicuous for thicker phantom.

**NEQ** ; For 2-cm-thick phantoms, the NEQ did not change with the tube voltage and filtration except for 32 and 35 kV with Rh filter. The NEQ values on these two exposure conditions were lower than that on others. Also, 35 kV with Rh filter gave the highest NEQ for phantom thicker than 4 cm. In these cases, the superiority of Rh filter to Mo filter became clearer for thicker phantom.

**SNR<sub>i</sub>** ; The SNR<sub>i</sub> is shown in Figure 2. For 2-cm and 4-cm-thick objects, we recognized the tendency that the SNR<sub>i</sub> value with Mo filter was larger than that with Rh filter. The SNR<sub>i</sub> increased when the tube voltage decreased.

#### Discussion

The results of WS and NEQ indicate that when the image contrast and AGD was constant, for a phantom thinner than 5 cm, an increase of the tube voltage did not improve the noise

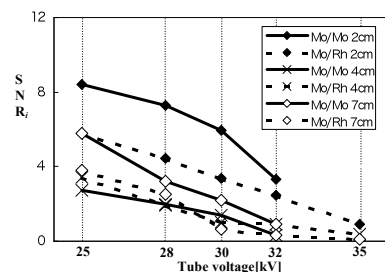


Fig.2 The results of SNR<sub>i</sub> for each thickness of phantoms

property of images very much. The results also showed that image property with the target/filter Mo/Rh was better than that with Mo/Mo for the phantom thicker than 4 cm. In general, it is said that high tube voltage delivers fine noise property. The result of our study contradicted this common theory on the x-ray energy level for mammography.

The result of SNR<sub>i</sub> differs from the results of WS and NEQ. In this study, the SNR<sub>i</sub> depends on the contrast of signal. We should make accuracy high with the low contrast object that has another intensity.

#### Conclusions

In conclusion, when the image contrast and AGD was constant, for the phantom thinner than 5 cm, an increase of the tube voltage did not improve the noise property of images very much. The results also showed that image property with the target/filter Mo/Rh was superior to that with Mo/Mo for the phantom thicker than 4 cm. Our result signified that high tube voltages did not improve the noise property of images with the x-ray energy level for mammography.

The result of SNR<sub>i</sub> conflicted with the results of WS and NEQ. We need to examine SNR<sub>i</sub> with the low contrast signal which have various intensity.

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## MULTI-MATERIAL DECOMPOSITION IMAGING IN THE PHOTON COUNTING DETECTOR

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#### Introduction

The photon counting detectors such as cadmium zinc telluride (CZT) and cadmium telluride (CdTe) have powerful advantages compared to energy integrating detectors. CZT or CdTe can detect individual gamma-ray or x-ray photon with energy discrimination. In recent years, energy-resolving and material decomposition x-ray imaging based on photon counting detectors has attracted attention from biomedical imaging researchers. One group has reported two contrast agents could be distinguished by the k-edge CT image using photon counting detectors.

Monte Carlo simulation is an essential tool in emission and transmission imaging that can assist in the design of new medical imaging devices, the optimization of acquisition protocols and the development or assessment of image reconstruction algorithms and correction techniques. This year, the OpenGATE collaboration has released GATE 6.0, supporting simulations of radiotherapy and computed tomography. They provide geometry of computed tomography with photon counting based readout.

We evaluated a large-area CZT detector originally built as a radionuclide detector for a small animal SPECT/CT system in combination of a microfocus x-ray source with a general goal of developing a material-decomposition imaging method. In addition, the characteristic study of two photon counting materials, CZT and CdTe were evaluated in the GATE environment.

#### Materials and Methods

The presentation will describe the basic principles of multi-material decomposition imaging based on photon counting detector. The experimental results from a feasibility study of multi-material decomposition imaging using the developed small animal SPECT/CT system will be presented. In addition, the simulation results for the characteristic of photon counting detector and multi-energy imaging of multi-material phantom will be presented.

#### Experimental feasibility of multi-material decomposition imaging

The characteristics of x-ray tube from Oxford Instruments (Scotts Valley, CA, USA) are: tungsten anode, source size 80 μm x 70 μm, maximum operating current 1 mA, and maximum accelerating voltage 60 kV. The 2 mm thick aluminum filter was used to attenuate x-rays with energies below the noise floor of the detector.

The large area photon counting CZT detector manufactured by Gamma Medica Ideas (Northridge, CA, USA) was used. The detector has an active detection area of 20 x 20 cm<sup>2</sup>, with multiple modules tiled together. The detector is configured as a 5 x 5 array of CZT modules, each with a 16 x 16 array of pixels and a pixel width of 2.5 mm. Thus, the used CZT detector is an 80 x 80 pixel array covering an active area of 20 x 20 cm<sup>2</sup> with a crystal thickness of 2.5 mm.

For the feasibility study of multi-material decomposition imaging a phantom that included various materials was built.

We designed the phantom using a 15 x 15 cm<sup>2</sup> piece of cellophane. We had attached 7 materials, with a range of densities and atomic numbers, on the cellophane. The materials are including glass (silicon dioxide), highly ordered pyrolytic graphite (HOPG), clay, titanium, plastic, aluminum, buffalo nickels, walking liberty half dollar coin.

The CZT detector was positioned 84.3 cm from the x-ray tube focal spot. The phantom was taped in contact with, and centered on, the CZT detector. The multi-material x-ray images were obtained at 50 kVp, 0.5 mA, and images were acquired for 140 sec/image. Acquired images corrected for bad pixels, gain, and offset. The attenuation of each material was obtained by the ratio of the emergent and incident radiation intensities I/I<sub>0</sub> according to the x-ray energy.

#### CZT detector in x-ray spectroscopy with different pixel sizes and pitches

GATE version 6.0 was used to perform an accurate simulation of the different pixel sizes. In this simulation study, charge sharing and pulse pileup were not considered. The Cd<sup>109</sup>ZnTe detector (x = 0.1, density = 5.68 g/cm<sup>3</sup>) used in this study. The CZT detector was 10x10 mm<sup>2</sup> and thickness of 3 mm. The thickness was chosen since it allows >90% absorption of the photons at 120 kVp. In an attempt to evaluate the detection ability, we designed the CZT detector with different pixel size on GATE. The pixels with sizes of 0.09 x 0.09, 0.45 x 0.45, and 0.90 x 0.90 mm<sup>2</sup> were simulated.

#### Energy resolved photon counting x-ray imaging with CZT detector

We designed a cylindrical PMMA phantom including three Iodines in concentration of 140, 40, and 8 mg/cm<sup>3</sup> and one Gadolinium in concentration of 39.3 mg/cm<sup>3</sup>. We studied the ability to separate different materials from the multi-energy windows. We obtained images from 23-32, 33-42, 40-49 and 50-59 keV energy windows. For each energy windows the contrast-to-noise ratio (CNR) was considered to be the image quality.

#### Results

Measured attenuation of each material was in a good agreement with the reference data of the NIST physics laboratory. The attenuation of x-rays in the various materials is dependent on the energy of the x-rays and the atomic composition of the material.

Three different pixel spectrums were distorted towards the lower energy region. Because the characteristic x-rays add counts in the range of 20-40 keV. The photoelectric effect is dominant at Cd and Te of CZT material due to the high Z numbers. The Z numbers of Cd and Te are 48 and 52. As characteristic x-rays of Cd and Te process quite high energy, they escape from crystals. Especially, these effects are evident in the small pixel sizes.

For Iodine, the CNR was found to dramatic increase in the range of 33-42 keV. For Gadolinium, the calculated value of

the CNR in the range of 50-59 keV was higher than those below 50 keV. Because the photons with energy levels just above the K-edge energy are more likely to be absorbed than photons with energy levels just below the K-edge energy.

**Conclusions**

We demonstrated that multi-material decomposition imaging is experimentally feasible using the photon-counting CZT detector and polychromatic x-ray. The photon counting based functional and anatomical imaging system will be designed in the GATE.

## DOSIMETRIC CHARACTERIZATION OF FLATTENING FILTER FREE LINAC USING MONTE CARLO SIMULATION

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**Introduction**

The flattening filter (FF) of medical linac is placed in the photon beamline to produce a flat beam profile at the given depth, but it decreases primary beam intensity by scattering and absorption. Recently some researches have shown that removing FF from medical linac can increase dose rate considerably, and reduce out-of-field doses [1-4]. In this study, we provide dosimetric properties of 6 MV and 10 MV photon beams from FF free (FFF) linac using Monte Carlo (MC) simulation.

**Materials and Methods**

*MC modeling of medical linac with and without FF:* We have developed 6 MV and 10 MV photon beam model for the linac head of Varian Clinac series accelerator using EGSnrc/BEAMnrc MC code [5]. The linac head components, including target, primary collimator, vacuum window, FF, monitor ion chamber, mirror, and X and Y jaws, were simulated on the basis of manufacturer-provided information. To calculate dose distributions in water phantom, we applied EGSnrc/DOSXYZnrc MC code [6].

The initial electron beam incident on the target have the distribution of the electron energy and the radial intensity assumed Gaussian in shape[7], and mean energy and intensity distribution of the initial electron is not clearly and varies among the same model of linacs. To determine electron energy, we comparing the measured and calculated percentage depth dose (PDD) for 30 x 30 cm<sup>2</sup> field size. After initial electron energy selection, we determined intensity distribution comparing the measured and calculated off-center ratio (OCR) at the depth of 10 cm for 5 x 5, 10 x 10, 20 x 20, and 30 x 30 cm<sup>2</sup> field sizes. To compare the measured and calculated dose, local dose difference were calculated by following equation:

$$Local\ dose\ difference = \left[ \frac{dose_{measurement} - dose_{MC}}{dose_{measurement}} \right]$$

The mean energy of the initial electron and the distributions of the electron energy and the radial intensity are shown in Table 1. Using determined initial electron, the phase-space (PS) file, containing data relating to particle position, direction, charge, and energy for 5 x 5, 10 x 10, 20 x 20, and 30 x 30 cm<sup>2</sup> field sizes at source surface distance (SSD) of 100 cm with and without FF were produced.

**Table 1 Initial electron parameters. The energy and intensity spread is given at FWHM**

Nominal energy (MV)	Derived energy (MeV)	Energy spread FWHM	Intensity spread FWHM (cm)
6	6.0	3%	0.15
10	10.5	3%	0.15

*Dosimetric properties of FFF linac:* To find out the characteristic of FFF linac, we compared PDD and OCR for varied field sizes, dose per incident electron, planer fluence, energy fluence, and energy spectrum with standard linac using EGSnrc/DOSXYZnrc and BEAMDP sub code [8].

**Results and Discussion**

*Validation of MC modeling:* To validate our MC simulation, calculated PDD and OCR were compared with measurement data. There were good agreement between MC and measurement results, and local differences were less than 3% in all region. The examples of calculated and measured PDD and OCR are shown in Fig. 1 and 2, respectively.

**Fig. 1 PDD for 10 x 10 cm<sup>2</sup> field size of 6 MV and 10 MV. Monte Carlo is circle and measurement is solid.**

**Fig. 2 OCR at the depth of 5 cm, 10 cm, 20 cm, and 30 cm for 10 x 10 cm<sup>2</sup> field size of 6 MV. Monte Carlo is circle and measurement is solid.**



# ESTIMATION OF RADIATION DOSES FROM X-RAY CT USING MONTE CARLO SIMULATION

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## Introduction

The purpose of this study is to calculate accurate radiation dose in patient from CT examination using voxel phantom. In the calculation, scanner-specific descriptions that include filtration designs, an orbital overlap and the absorption of CT table are necessary. A special filter called "beam-shaping filter" which is positioned in front of the x-ray tube of the CT units causes less uniform x-ray fan beam (Figure 1). We took into account the effect of the filter based on measured data in this simulation.

A standard Japanese Model can be developed by using the voxel phantom from CT DICOM (Digital Imaging and Communication in Medicine) format data. In-phantom dosimetry system, as will hereinafter be described in detail, was used to compare the measurements and the calculations.

## Materials and Methods

### 1. Overview

All simulations were performed using the EGS5 (Electron Gamma Shower ver.5) Monte Carlo code, which is a general purpose package for the Monte Carlo simulation of the coupled transport of electrons and photons in an arbitrary geometry for particles with energies above a few keV up to several hundred GeV. For measurements and simulations, a Non-helical X-ray CT unit TCT-300 (Toshiba Medical Systems, Tochigi, JAPAN) was used in this study. The x-ray tube voltage was 120kV.

### 2. Effect of beam-shaping filter

A typical CT scanner is equipped with x-ray beam filtration that includes the beam-shaping filter. This filtration modifies a number of x-ray beam properties (effective energy, flux, first and second order statistics), making them non-uniform across the fan beam. The beam-shaping filter is used to adjust the beam quality of x-rays after passing through a patient. There is no such published data on the design of beam-shaping filter, which may vary considerably from scanner to scanner. We measured aluminum Half Value Layer (AL HVL) and dose value after passing through the beam-shaping filter and incorporated measured values in the simulation. New type of semiconductor dosimeter called Rapidose (Radcal, Monrovia, CA) was used for the measurements. Rapidose has functions that can measure dose and AL HVL at the same time and it is suitable for measurement of relatively high energy x-ray such as x-ray CT. In the measurement of the effect of beam-shaping filter, x-ray tube was fixed on the 0:00 position on its orbital. Along a fan beam of x-ray CT, Rapidose was moved up to 19 degrees measured at every one degree. In the simulation, photon number at each beam angle along the fan beam was determined by dose profiles and energy spectrums were generated by Birch's formula based on AL HVL measured on each angle was used.

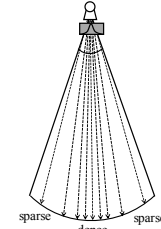


Fig.1 Incident photon number at each beam angle

### 3. Specific factors related to the CT device

There are several specific factors that are related to the CT scanner. Non-helical CT scanner has x-ray tube orbital overlap that cause the positions of the x-ray beam overlap on the patient. The overlap angle was adjusted to match the measured CTDI values in the simulation because the information of the actual overlap angles wasn't published by the manufacturer. A full of x-ray tube rotation was estimated at 376 degrees including overlap of +/-8 degrees.

The table top is usually made of carbon fiber due to its strength and low x-ray attenuation properties. In this simulation, the CT table was composed of density adjusted carbon. The density of the carbon was adjusted to correspond with the actual dose ratio table(+)/(-). These factors were also incorporated in the simulation code.

### 4. Measurement and calculation of CTDI

Computed Tomography Dose Index (CTDI) measured by using a CT acrylic phantom was used for comparison between the measurement and the calculation. The CTDI phantom is 15 cm in length with a diameter of 30 cm. CTDI values are shown in air kerma (kinetic energy released in matter; mGy) in the phantom's center hole and four peripheral holes (0°, 90°, 180°, 270°). In order to determine the air kerma from CT examination, simulated deposit energies in units of MeV/gram/source-particle were converted to air kerma in units of mGy by a conversion factor ( $C_f$ ) [1]. An in-air normalization method that is based on pencil-chamber exposure reading for a single axial scan was taken at the isocenter of the CT gantry free in air. The conversion factor was defined as

$$C_f = \frac{(CTDI_{100,air,measured\ per\ mAs})}{(CTDI_{100,air,simulated\ per\ particle})} \quad (1)$$

where  $CTDI_{100,air,measured\ per\ mAs}$  is measured by an ion chamber free in air of the x-ray CT isocenter, and  $CTDI_{100,air,simulated\ per\ particle}$  is obtained by simulation.

The absorbed dose  $D_{ab}$  is calculated by

$$D_{ab} = D_{sim} \times C_f \quad (2)$$

where  $D_{sim}$  is the calculated dose in the simulation.

**Dosimetric properties of FFF linac:** We considered dose per initial electron at the depth of 10 cm for dose rate comparison. For  $10 \times 10 \text{ cm}^2$  field size, the ratios of dose per initial electron for FFF linac to standard linac were 2.38, 5.17 for 6 MV, 10 MV, respectively.

The depth dose and OCR were calculated for field sizes of  $5 \times 5$ ,  $10 \times 10$ ,  $20 \times 20$ , and  $30 \times 30 \text{ cm}^2$ . The depth dose curves of FFF linac for  $10 \times 10 \text{ cm}^2$  field for 6 MV and 10 MV, are shown in Fig. 3 and 4, respectively. The depth dose was normalized at maximum point of measurement with FF. In the beyond maximum dose region, the dose for standard linac is higher than that for FFF linac due to the beam hardening effect of the FF. In the build up region, because of softer spectra and contamination low-energy electron, FFF linac delivers higher dose than standard linac. Especially, for 10 MV, significant increasing dose in the build up region is observed due to the excessive contamination electron. It seems that more penetrating electron through the target reach the phantom for 10 MV than for 6 MV, because the target of 6 MV is tungsten and copper, but that of 10 MV is copper only.

fluence was reduced with distance from central axis as a result that removing FF reduced scattering radiation from linac head.

Fig. 5 Comparison of OCR at the depth of 10 cm for with and without FF for  $10 \times 10 \text{ cm}^2$  field size of 10 MV. without FF, MC is circle and with FF, measurement is solid.

## Conclusions

The MC model of Varian Clinac series accelerator with and without FF were developed and compared. Our results shows that removing FF can increase dose rate and decrease out-of-field dose. These could be an advantage of FFF linac, but for clinical usage, FFF linac should equip the filter to remove the excessive contamination electron.

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Fig. 3 Comparison of depth dose for with and without FF for  $10 \times 10 \text{ cm}^2$  field size of 6 MV. without FF, MC is circle and with FF, measurement is solid.

Fig. 4 Comparison of depth dose for with and without FF for  $10 \times 10 \text{ cm}^2$  field size of 10 MV. without FF, MC is circle and with FF, measurement is solid.

The OCR at the depth of 10 cm of 10MV FFF linac for  $10 \times 10 \text{ cm}^2$  is shown in Fig. 5. OCR was normalized at the central axis dose. By removing of FF, the dose was reduced with distance from the central axis. In addition, removed FF reduced the out-of-field dose. For example, in Fig. 5, at a distance of 7 cm from the central axis, the ratio of doses for FFF linac to standard linac was 0.8. This is because photon

## 5. Development of voxel phantom

Two different types of voxel phantoms were developed based on CT image. One of them (THRA-1, Kyoto Kagaku Co., Kyoto, JAPAN) is only composed of lung, bone and soft tissue but can obtain organ doses using a number of internal semiconductor dosimeter installed at the position of tissues and organs [2]. The semiconductor dosimeter is implanted in the centroid of the organ and tissue. This system is called "in-phantom dosimetry system" and was used to compare measurements and calculations. Measurements and calculations were performed on thoracic scan protocol. The resolution of the axial simulation matrix was decreased from  $512 \times 512$  to  $170 \times 170$  to decrease runtime of the simulation. The size of each voxel was  $0.1875 \times 0.1875 \times 1.0 \text{ cm}^3$ .

Another phantom called CTU-41 (Kyoto Kagaku Co., Kyoto, JAPAN) includes the shapes of organs with individual densities. The average radiation dose to each organ was estimated in CTU-41 phantom. Both "in-phantom dosimetry system" and CTU-41 phantom were scanned by x-ray CT, and image data was output as DICOM format. The organs and tissues were assigned in each voxels using digital value (Hounsfield Unit).

## Results and Discussions

### 1. Effects of the beam-shaping filter

Figure 2 shows the dose distributions and energy changes along the beam angle. The effective energy of x-ray beam determined using the measured AL HVL. Relative dose value maintained the same level from 0 degree to  $\pm 4$  degree, and after that it rapidly decreased as the angle became bigger. Similarly, effective energy maintained up to  $\pm 4$  degree, and then it rapidly increased as the angle became bigger and was saturated up from  $\pm 9$  degrees. The cause of the effect was that the thickness of the filter was bigger as the beam angle was bigger.

### 2. Comparison of CTDI between measurements and calculations

Table 1 shows comparison of measurements and calculations of the CTDI. The agreement between measurements and calculations was within 2.8%. The CTDI at  $0^\circ$  position was highest due to x-ray tube orbital overlap and lowest at  $180^\circ$  position due to absorption of the CT table. The simulated CTDI values were approximately equal to measured CTDI values. With this result, the x-ray source and the geometry of x-ray CT could be incorporated in the simulation successfully.

### 3. The result of calculation using voxel phantom

The result of the comparison of measurement and calculation of the in-phantom dosimetry system is shown in Figure 3(a). Locations of the calculation point were accorded with that of semiconductor dosimeters in the phantom. Measurement and calculation shown the same tendency on the same location in the phantom, but calculated values were generally slightly greater than measured values.

Figure 3(b) shows comparison of the organ doses between in-phantom dosimetry system and CTU-41 phantom. Dose values of in-phantom dosimetry system were measured

value, those of CTU-41 phantom were converted from deposited energy for each of whole organ by simulation. Calculated dose of the organ in the scan area such as lung was approximately equal to measurement dose, but the organ dose of proximity to the end of scan area such as thyroid resulted in significantly different from measured dose. In the calculation, the deposited energy in whole organ was divided by the organ mass and this value was presented as an average organ dose. When the organ was partially irradiated in the end of the scan area, average organ dose was smaller than the whole-organ irradiated case. In the measurement, the organ dose was presented maximum value as is the case in whole-organ irradiation if the evaluated point as a centroid of the organ fell within the scan area.

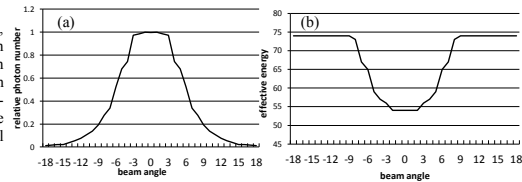


Fig.2 Relative dose and effective energy measured by Rapidose at each beam angle. (a)The relative dose at each beam angle. (b)The effective energy calculated by measured Half Value Layer at each beam angle.

Table 1 Comparison of measurements and calculations of the CTDI.

position of the dosimeter	measurement [mGy]	simulation [mGy]	difference [%]
top	21.0	20.8	-1.2
center	11.7	11.5	-1.4
left	16.6	16.5	-0.4
right	16.0	16.5	2.8
bottom	14.8	14.5	-2.4

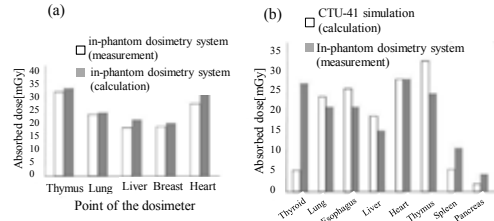


Fig.3 Comparison of measurement and calculation (a) point dose evaluation with same phantom (b) whole organ dose versus point dose

## References

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# EVALUATION OF THE DISTRIBUTION OF INTRACRANIAL NAVIGATION ACCURACY IN IMAGE-GUIDED NEUROSURGERY

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## Introduction

Nagoya University Hospital has introduced a neuronavigation system for accurate resection of malignant brain tumors. Surgeons can confirm the positional relationship between tumors and normal tissue by using this system. Especially, this system is very useful in the resection of tumors located near the functional areas.

Accuracy of a neuronavigation system is very important to ensure a safe and accurate resection [1]. Various factors influence the navigation accuracy [2]; one of the important factors is the configuration of the fiducial markers that are positioned on the patient's head. A fiducial marker is the reference point of the navigation system (Fig. 1). The configuration of fiducial markers varies among institutions, and it can influence intracranial navigation accuracy. In this study, we investigated the effect of the configuration of fiducial markers on the distribution of intracranial navigation accuracy.



Fig. 1 Fiducial marker (doughnut shape)

A fiducial marker is used as a reference point of the navigation. They are positioned on the patient's head before surgical operation.

## Materials and Methods

We evaluated intracranial navigation accuracy for 2 different configurations of fiducial markers (the clustered and scattered patterns) by using a skull-shaped phantom (Fig. 2). Because this phantom structurally resembles the human head, intracranial navigation accuracy can be measured in keeping with the clinical situation. We also measured the navigation accuracy by varying the number of fiducial markers. We set 4, 5, or 6 markers on the surface of the phantom for each configuration. In the clustered configuration, the markers were placed on the frontal, temporal, and occipital bones. In the scattered pattern, they were placed on the forehead, temple, and mastoid regions, same as Nagoya University Hospital's method. To measure the navigation accuracy, fiducial markers were positioned on the bottom of the phantom. And to measure the navigation accuracy on the central portion of the phantom, 5 columns and 2 circular plates were constructed inside the phantom. We can investigate the distribution of the navigation accuracy by

using 27 fiducial markers positioned inside the phantom (Fig. 3). The phantom was filled with nickel chloride, and magnetic resonance images were acquired. MR imaging was performed with 0.4T MRI (APERIO Inspire; Hitachi Medical Co., Chiba, Japan). MR images were transferred to a planning system for navigation (iPLAN cranial; BrainLAB AG, Heimstetten, Germany), and registration points were set up on each fiducial marker positioned surface of the phantom. Registration is performed before surgery and is the process of integrating image-space coordinates with real-space coordinates. The steps of registration are as follows: (1) The operator registers the center of the fiducial markers as registration points by using the iPLAN software. (2) Registration is completed by pointing the center of each fiducial marker by using a navigation pointer. After registration was carried out using a navigation system (VectorVision Compact; BrainLAB AG), intracranial navigation accuracy was measured.

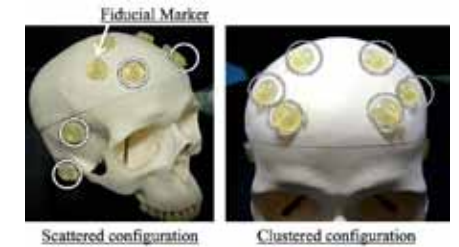


Fig. 2 Skull-shaped phantom with fiducial markers

For the scattered configuration, the fiducial markers were placed on the forehead, temple, and mastoid regions (left). For the clustered configuration, the fiducial markers were placed on the frontal, temporal, and occipital bones (right).

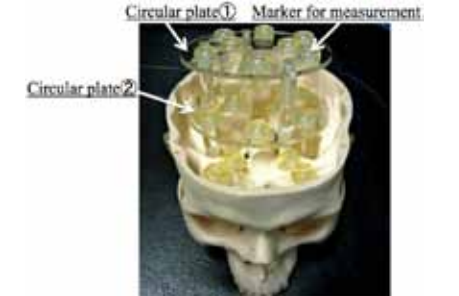


Fig. 3 Measurement point of the phantom

We can investigate the navigation accuracy by using 27 fiducial markers positioned inside the phantom. Eight fiducial markers

were positioned on each circular plate. Also, 4, 2, and 5 fiducial markers were positioned on the anterior, middle, posterior cranial fossa respectively.

**Results**

The mean intracranial navigation accuracy for the clustered pattern with markers placed on the frontal bone was  $2.13 \pm 1.15$  mm with 4 markers,  $1.92 \pm 1.28$  mm with 5 markers, and  $2.01 \pm 1.02$  mm with 6 markers. The mean intracranial navigation accuracy for the scattered pattern was  $1.51 \pm 0.88$  mm with 4 markers,  $1.68 \pm 0.75$  mm with 5 markers, and  $1.24 \pm 0.37$  mm with 6 markers (Table 1). The scattered patterns were significantly better than the clustered pattern ( $p < 0.05$ ). We calculated the navigation accuracy on the central portion of the phantom and bottom of the phantom separately (Table 2). The accuracy for the clustered pattern with 6 markers was  $1.60 \pm 0.53$  mm on the central portion and  $2.59 \pm 1.26$  mm on the bottom of the phantom ( $p < 0.05$ ). The clustered pattern could maintain the accuracy only within a narrow region near the fiducial markers positioned on the surface of the phantom. In addition, the clustered patterns with markers positioned on the temporal and occipital bones indicated a similar tendency. However, the accuracy for the scattered pattern with 6 markers was  $1.17 \pm 0.37$  mm on the central portion and  $1.37 \pm 0.35$  mm on the bottom of the phantom, and there was no significant difference. Thus, the scattered pattern could maintain the accuracy over a wide region of the phantom.

**Table 1** Intracranial navigation accuracy

Configuration	Number	The mean ( $\pm$ SD) navigation accuracy (mm)
Clustered	4	$2.13 \pm 1.15$
	5	$1.92 \pm 1.28$
	6	$2.01 \pm 1.02$
Scattered	4	$1.51 \pm 0.88$
	5	$1.68 \pm 0.75$
	6	$1.24 \pm 0.37$

**Table 2** Navigation accuracy on the central and bottom of the phantom

Configuration	Number	Central portion ( $\pm$ SD) (mm)	Bottom ( $\pm$ SD) (mm)
Clustered	4	$1.72 \pm 0.59$	$2.72 \pm 1.47$
	5	$1.36 \pm 0.59$	$2.73 \pm 1.56$
	6	$1.60 \pm 0.53$	$2.59 \pm 1.26$
Scattered	4	$0.96 \pm 0.14$	$2.32 \pm 0.89$
	5	$1.39 \pm 0.52$	$2.10 \pm 0.83$
	6	$1.15 \pm 0.37$	$1.37 \pm 0.35$

**Discussion**

The configuration of the fiducial markers varies among institutions and it influence the distribution of the navigation accuracy. It is difficult to measure intracranial navigation accuracy in clinical practice [1]. Therefore, we evaluated the effect of the configuration of the markers on the navigation accuracy by using a skull-shaped phantom. Because this phantom structurally resembles the human head, intracranial navigation accuracy can be measured in keeping with the clinical situation.

The clustered pattern, regardless of the installation location, could maintain the navigation accuracy only within the narrow region near the fiducial markers. The navigation accuracy decreased with an increase in the distance from the fiducial markers positioned on the surface of the phantom. However, the scattered pattern showed high accuracy on the entire phantom, with two exceptions: the scattered pattern with 4 and 5 markers could not maintain high accuracy on the bottom of the phantom because fiducial markers positioned on the mastoid were not used. To maintain a high accuracy on the entire phantom, it is necessary to position the fiducial markers across a wide area on the head. If a tumor exists on the base of skull or if the tumor is huge, the scattered configuration is appropriate for safe surgery.

**Conclusions**

In this study, we evaluated the effect of the configuration of the fiducial markers on the distribution of the navigation accuracy by using a skull-shaped phantom. It is important to position the fiducial markers across a wide area on the head for accurate resection of the tumor.

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Medical Technology (13:30 ~16:00 THP Seminar Room)

Session chairs: Prof. Shinya Wakusawa  
Prof. Tsutomu Kawabe

## Sleep disorder and sleep-disordered breathing in the elderly

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### Introduction

The elderly spend more time in bed, experience more awakenings during the night,<sup>1)</sup> and have increased complaints of sleep disorder such as the difficulty initiating sleep, difficulty remaining asleep and early morning awakenings.<sup>2)</sup> Elderly individuals complain primarily about sleep disorder, which are often secondary to other disorders.<sup>1)</sup>

Aging substantially increases the risk of obstructive sleep apnea syndrome (OSAS),<sup>3)</sup> and OSAS is associated with hypertension, cerebrovascular disorder, cardiovascular disease and all-cause mortality.<sup>4)</sup> However, effects of aging on pathophysiology of apnea/hypopnea in clinical settings have not been clearly investigated.

We assessed the sleep disorder in middle-aged and elderly people, and examined the relation between sleep disordered-breathing (SDB) and cardiovascular function in the elderly.

### Materials and Methods

**Subjects:** 32 middle-aged subjects (51.3±4.1 years, male: 16 subjects, female: 16 subjects) and 20 elderly (71.7±5.8 years, male: 5 subjects, female: 15 subjects) were targeted. The study protocol was approved by the appropriate institutional review committee at the Nagoya University, and informed written consent from the subjects was obtained at the beginning of the assessment.

**Functional Outcome Measures:** Subjective sleep symptoms, disturbances, and patterns were assessed with the Pittsburgh Sleep Quality Index (PSQI). The total global score ranges from 0 to 21, and greater scores indicate higher levels of sleep symptoms.<sup>5)</sup> Subjects with PSQI ≥ 6 were defined as insomnia. Daytime sleepiness was quantified using the Epworth Sleepiness Scale (ESS). Possible scores ranges from 0 to 24, and higher scores reflect greater sleepiness. A cut-off value ≥ 11 indicated excessive daytime somnolence (EDS).<sup>6)</sup>

**Actigraphy:** The actigraph (Ambulatory Monitoring, Inc, USA) was worn around the wrist of a non-dominant hand. The actigraph data were assessed using the algorithm supplied by the ActionW-2 clinical sleep analysis software package for Windows (Ambulatory Monitoring, Inc, USA). Sleep and activity were scored according to the Cole-Kripke formula.<sup>7)</sup> Total sleep time (TST) was measured using actigraphy.<sup>8)</sup>

**Measurements for Sleep Apnea:** To assess the sleep apnea, overnight examination was performed on each subjects using the portable home monitoring (apnomonitor: CHEST) and we calculated apnea-hypopnea index/h (AHI/h), oxygen desaturation index/h (ODI/h) and lowest arterial oxyhemoglobin saturation (Lowest SpO<sub>2</sub>). We determined the subjects of AHI ≥ 5/h as sleep apnea syndrome (SAS).

**Markers of Atherosclerosis:** We evaluated atherosclerosis from Pulse Wave Velocity (PWV) and carotid ultrasonography. PWV was measured using a volume-plethysmographic (from PWV/ABI; Nihon Colin Co.,Ltd., Komaki, Japan), which simultaneously records brachial-ankle PWV (baPWV), blood pressure, an electrocardiogram, and heart sounds.<sup>9)</sup> The intima-media thickness (IMT) of the carotid arteries was estimated by B-mode ultrasound (HEWLETT PACKARD SONOS 2000 : PHILIPS) with a 7.5MHz.<sup>10) 11), 12)</sup>

**Echocardiograph:** Standard echocardiography including measurement of transmitral flow velocity indices (HEWLETT PACKARD SONOS 2000 : PHILIPS) was performed in all subjects. Left atrium dimension (LAD), left ventricular end-diastolic dimension (LVDd) and left ventricular end-systolic dimension (LVDs) were measured by two-dimensional M-mode, and the left ventricular ejection fraction (LVEF) was calculated. The peak early velocity (E) and atrium contraction velocity (A), their ratio (E/A) and the deceleration time of peak E velocity (DcT) were measured from mitral inflow velocities.

**Data analysis:** All statistical analyses were performed using a statistical software package (Statview for Windows version 4.54 SAS Institute Inc: Cary, NC). Data were presented by mean ± standard deviation (SD), and analysis of variance follow (ANOVA) by Scheffe's test was used to compare means of continuous values between subgroups. A probability (p) value < 0.05 were considered statistically significant.

### Results

ESS was significantly higher in the middle-aged group than in the elderly group (8.1±5.1 vs 3.9±2.9, p=0.01). (Figure 1)

Bed time was significantly earlier in the elderly group than in the middle-aged group. (21.25±0.19 vs 22:59±0.17, p=0.019) There was no significantly difference between two groups in wake-up time. (Figure 2)

The prevalence of PSQI ≥ 6 (insomnia) was 45% in the elderly group, and it was 28% in the middle-aged group. In the elderly, Time in bed was significantly extended in the insomnia group than in the non-insomnia group. (8.6±1.6h vs 7.5±1.0h, p=0.038) (Table 1)

There were no significant differences between SAS and non-SAS groups in atherosclerotic and echocardiographic parameters. (Table 2)

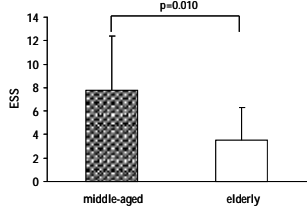


Figure 1. ESS between middle-aged and elderly group  
ESS: Epworth Sleepiness Scale

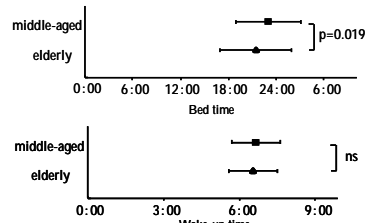


Figure 2. Bed time and wake-up time between middle-aged and elderly group

Table 1. Time in bed in the elderly group

	insomnia n=9	non-insomnia n=11	P value
PSQI	7.2±2.4	4.3±1.2	<0.001
Time in bed (hr)	8.6±1.6	7.5±1.0	<0.05

Data are presented as mean ± SD.  
PSQI: Pittsburgh Sleep Quality Index

Table 2. Atherosclerotic and echocardiographic parameters in the elderly group

	SAS n=11	non-SAS n=9	P value
R-IMT Max (mm)	0.9 ± 0.4	1.1 ± 0.5	ns
L-IMT Max (mm)	0.9 ± 0.5	1.0 ± 0.3	ns
R-baPWV (cm/s)	1608.0 ± 382.0	1568.0 ± 285.0	ns
L-baPWV (cm/s)	1628.0 ± 300.0	1597.0 ± 256.0	ns
LVEF (%)	66.4 ± 6.1	70.0 ± 12.1	ns
E/A	0.8 ± 0.2	0.9 ± 0.3	ns
DCT (sec)	207.0 ± 36.5	210.0 ± 59.4	ns

Data are presented as mean ± SD.  
IMT: intima-media thickness; baPWV: brachial-ankle pulse wave velocity; LVEF: left ventricular ejection fraction; E/A: peak early systolic velocity (E)/atrial contraction velocity (A); DCT: deceleration time of peak E velocity

## Discussion

Epidemiological studies have implicated OSAS as a risk factor for the development of cardiovascular and cerebrovascular disease. However, in this study, cardiovascular function did not significantly differ between SAS and non-SAS groups in the elderly. Moreover, AHI did not significantly change with a presence of hypertension or hyperlipidemia.

ESS score was significantly higher in the middle-aged group than in the elderly group, and bed time was significantly later. Daytime sleepiness was found in the middle-aged group and TST during the night was short. In the elderly, daytime sleepiness was not detected, but insomnia was permitted especially who spend long time in bed. Sleep disorder may relate to decrease of daytime activity and sleep quality.

In conclusion, comparison of sleep disorder/SDB and cardiovascular function between middle-aged and elderly population may provide insight into the pathophysiology of sleep disorder/SDB associated with aging.

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## Mycobacterium tuberculosis-induced expression of Granulocyte-Macrophage Colony Stimulating Factor is mediated by ERK1/2 MAPK or PI3-K/Akt signaling pathway

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## Introduction

*Mycobacterium tuberculosis*, the causative agent of tuberculosis, has already infected nearly a third of the world's population causing approximately 2 million deaths yearly. Infection with MTB affects the recruitment and activation of circulating effector leukocytes by influencing induction and secretion of cytokines from infected macrophages. Infected macrophages release a variety of inflammatory cytokines as defense mechanisms against MTB. The cytokine GM-CSF plays an important role in the differentiation of monocytes, alveolar macrophages and dendritic cells. GM-CSF acts locally as a proinflammatory cytokine. In the lungs, GM-CSF is very important for macrophage maturation and differentiation and host defense. It was previously reported that GM-CSF can induce up-regulation of MHC class II and costimulatory molecules such as CD80 and CD86 on antigen presenting cells (APCs) as well as increasing their phagocytic activity and stimulatory capacity. When this cytokine is lacking, as in gene-disrupted mice, the architecture of the lung is altered and alveolar macrophages become foamy in appearance. In addition, these cells have defects in phagocytosis, bacterial killing, as well as loss of Toll-like receptor expression and signaling. In the context of tuberculosis, GM-CSF may also contribute to the cytokine/chemokine milieu responsible for granuloma formation in the lung. Over-expression of GM-CSF in the lungs impairs protective immunity against MTB and therefore careful regulation of pulmonary GM-CSF levels may be critical in sustaining protection against chronic tuberculosis disease. GM-CSF act as a pivotal factor for the development of alveolar macrophages in lung. Increasing GM-CSF levels into the chronic stage of the infection results in elevated cell proliferation and phagocytic activity. GM-CSF regulates both pulmonary surfactant homeostasis and the differentiation and proliferation of functionally competent alveolar macrophages. But the intracellular signaling pathways involved in the regulation of GM-CSF expression in alveolar macrophages are unclear. In this study, we aimed to elucidate the involvement of GM-CSF in the host immune response against MTB infection. We observed mycobacterium-stimulated induction of GM-CSF and evaluated the associated with signal transduction pathway.

## Materials and Methods

### Preparation of Mycobacteria

*Mycobacterium tuberculosis* H37Rv (ATCC 27294), *M. bovis* BCG (ATCC 35748) and clinical isolated Beijing family of MTB strain (K-strain) obtained at Yonsei Medical school in Seoul and used in this study was grown for about four weeks at 37°C as a surface pellicle on Sauton medium enriched with 0.4% sodium glutamate and 3.0% glycerol. The surface pellicles were collected and disrupted by gentle vortexing with 6mm glass beads. After clumps had settled, the upper suspension was collected and aliquots were stored at -80°C. Before infection,

aliquots were thawed and quantitated for viable colony-forming units (CFU) on Middlebrook 7H10 agar (Difco, USA)..

### Cell culture and infection of mycobacteria

THP-1 cell line was maintained in RPMI 1640 medium with 2 mM glutamine, 10% heat inactivated fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco-BRL, USA) at 37°C under 5% CO<sub>2</sub> condition. THP-1 cells were seeded in six-well plates and treated with 50 nM phorbol-12-myristate-13-acetate (PMA; Sigma) for 48 h to induce differentiation into macrophage-like cells, then washed three times with antibiotics free RPMI 1640 medium. Before infection, differentiated THP-1 cells were reconstituted in antibiotics free RPMI 1640 medium with 5% FBS. Cells were incubated with MTB at 10 MOI for 0, 1, 1.5, 3, 6, 9, 12, 18, 24, 48 or 72 h. PMA-differentiated THP-1 cells were pretreated with inhibitors for 45 min before stimulation with MTB H37Rv for 4 h at 10 MOI.

### RNA extraction and Semi-quantitative RT-PCR

Total RNA was extracted from cultured cells using Trizol Reagent (Invitrogen, USA) according to the manufacturer's instruction. cDNA was synthesized by reverse transcription with 2 µg total RNA, 0.25 µg of random hexamer (Invitrogen) and 200 unit of Murine Molony Leukemia Virus Reverse Transcriptase (MMLV-RT; Invitrogen) for 10min at 25°C, 50min at 37°C and 15min at 70°C. Diluted reverse-transcribed cDNA was used subsequent 25 µl PCR amplification. PCR amplification using 0.2 units of G-Taq polymerase (Cosmo Genetech, Korea) was performed in a thermocycler (Applied Biosystems, USA) for 35 cycles using GM-CSF primer. GAPDH was amplified as an internal control. The intensity of each band amplified by RT-PCR was analyzed using Gel Doc EQ Quantity One (Bio-rad, Italy) and normalized to GAPDH mRNA in corresponding samples.

### Enzyme-linked immunosorbent assay (ELISA)

Cell culture supernatants from MTB-infected THP-1 cells were collected 24 h after infection. Cell culture supernatants were analyzed using Human GM-CSF ELISA MAX™ Standard (BioLegend, USA), as recommended by the manufacturer.

### Statistics

All values are given as mean ± standard deviation (SD). When a significant difference was detected, further analysis was performed using a Student's t-test. A p value of less than 0.05 was considered significant.

### Results

MTB infected THP-1 cells induced a dose-dependent manner and a time-dependent increase in GM-CSF mRNA

Expression of GM-CSF was up-regulated by infection of MTB in a dose-dependent manner, reaching a peak at 20 MOI of MTB. We also examined the effects of mycobacterial infection on the timing of GM-CSF expression. GM-CSF expression was up-regulated in response to MTB in a time-dependent manner. The mRNA level of GM-CSF peaked at 6 h after mycobacterial infection, and then declined gradually for 72 h.

*Induction of GM-CSF by MTB is mediated by p38 MAPK, ERK1/2 MAPK, PI3-K or PLC signaling pathway.*

To elucidate the mechanism by which mycobacterial infection affects expression of GM-CSF, we determined the signaling pathway associated with MTB-stimulated induction of GM-CSF. Treatment with Ro-31-8425 (an inhibitor of calssical PKC) or SP600125 (an inhibitor of JNK) did not influence of GM-CSF induction by MTB. SB202190 (an inhibitors of p38 MAPK), PD98059 (inhibitor of ERK1/2), Ly294002 (inhibitor of PI3-K) and U73122 (inhibitor of PLC) had negative effects on MTB-stimulated GM-CSF expression.

*Mycobacterial infection induces GM-CSF secretion and the induction of GM-CSF by MTB is mediated by ERK1/2 MAPK or PI3-K/PDK1/Akt signaling pathway.*

Differentiated THP-1 cells were pre-treated with indicated concentrations of PD98059 (ERK1/2 inhibitor), Ly294002 (PI3-K inhibitor), OSU03012 (PDK1 inhibitor) or Akt (Akt inhibitor IV) for 45 min, followed by MTB infection (10 MOI). Supernatants were harvested 24 h after infection and secretion of GM-CSF was measured by ELISA. All signal inhibitors were decreased GM-CSF secretion at high dose of specific signal inhibitors. PD98059 and Ly294002 decreased about 85 % of GM-CSF secretion, OSU03012 and Akt decreased about half of GM-CSF secretion.

#### Discussion

The Members of the CSF cytokine family play important roles in macrophage recruitment and activation to regions of inflammation. In mycobacterial infection, alveolar macrophage displays increasing levels of GM-CSF. However, mechanism of GM-CSF induction does not poorly understood. We investigated whether increasing levels of GM-CSF in mycobacteria infected THP-1 cell mediated mycobacterial secreted proteins. The increase in expression and secretion of GM-CSF was caused by MTB was reduced in cells treated with inhibitors of ERK1/2 MAPK. In addition the induction of GM-CSF caused by MTB was partially reduced in cells treated with inhibitors of p38 MAPK. In addition, the increase in secretion of GM-CSF caused by MTB was reduced in cells treated with inhibitors of PI3-K, PDK1 and Akt. These results suggest that GM-CSF plays important role in the immune response against mycobacterium infection. Therefore this signal transduction system might be a target for screening therapeutic candidates for mycobacterial disease

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## MOLECULAR BASIS OF DECREASE OF PROTEIN S IN PREGNANCY: 17 $\beta$ -ESTRADIOL DOWN-REGULATES PROTEIN S (PS) EXPRESSION RECRUITING CO-REPRESSOR COMPLEX TO THE PS PROMOTER

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#### Introduction

Anticoagulant Protein S (PS) is a vitamin K-dependent plasma protein that functions as a nonenzymatic cofactor for activated protein C in the anticoagulation pathway [1]. Decreased levels of plasma PS are known as a risk factor for the development of deep venous thrombosis, and there are many factors to cause the PS deficiency [2-4].

Hereditary PS deficiency is determined by detection of genetic mutation in PS $\alpha$  gene (*PROS1*) [5]. PS deficiency can also occur throughout life under the acquired conditions such as oral anticoagulant (i.e. warfarin) use and liver disease [6]. Furthermore, acquired PS deficiency has been reported in individuals with high levels of estrogen during pregnancy and in those taking oral contraceptives [7-9].

Estrogens are reported as the regulator of the specific gene expression via estrogen receptors [10]. The estrogen receptor (ER) is one of the steroid/nuclear receptor, and the ligand-bound ER functions as a transcriptional factor [11]. The classical ER action involves ligand-induced formation of an ER homodimer, which interacts with estrogen responsive elements (EREs) in target gene promoters [11]. The ligand-occupied ER is also known to associate with other transcription factors, not mediating EREs to modulate ligand-dependent gene expression [12-15].

In this study, we investigated the molecular basis of the PS decrease caused by E2, and thus the genomic ER signaling pathway modulating ligand-dependent *PROS1* gene repression, in HepG2-ER $\alpha$  cells stably expressing human ER $\alpha$ .

#### Materials and Methods

*Establishment of HepG2-ER $\alpha$  cell line:* HepG2 cells that stably express human ER $\alpha$  (HepG2-ER $\alpha$ ) was established by G418 selection and used for further study.

*Quantitative RT-PCR and ELISA:* Quantitative reverse transcription (RT)-PCR was performed to determine PS mRNA and GAPDH mRNA using Power SYBR Green Master mix. Enzyme-linked immunosorbent assay (ELISA) was carried out to determine human PS antigen.

*Luciferase Reporter Assay:* To determine the *PROS1* promoter activity, luciferase reporter assay was performed in respective cell lines. Luciferase activity was normalized to the activity of co-transfected  $\beta$ -galactosidase.

*Electrophoretic Mobility Shift Assay (EMSA), DNA Pull-down Assay and Chromatin Immunoprecipitation (ChIP) assay:* EMSA and DNA pull-down assay were carried out to determine the transcription factors binding to the *PROS1* promoter regions using artificial double-stranded oligonucleotides. ChIP assay was performed with specific antibodies against various transcription factors bound intracellular DNA of the cells.

#### Results

*PS expression was repressed by 17 $\beta$ -estradiol in human normal hepatocytes and HepG2-ER $\alpha$  cells:* To determine the effect of 17 $\beta$ -estradiol (E2) to the *PROS1* expression, we analyzed PS mRNA in human normal hepatocytes (hNHeps) and HepG2 or its-derived cell lines. In ER $\alpha$ -positive hNHeps treated with 100nM E2, PS mRNA was decreased compared with vehicle-treated cells. In HepG2-ER $\alpha$  cells, PS mRNA and antigen were also repressed by E2 treatment, but not in ER $\alpha$ -negative other cell lines.

*Down-regulation of PROS1 promoter activity by E2:* To investigate of the effects on *PROS1* promoter activity, we cloned the promoter region of *PROS1* and constructed luciferase-reporter vectors. In the full-length promoter construct (PS-4229/pGL3) and its-truncated constructs (PS-1826/pGL3 to PS-175/pGL3), luciferase activities were down-regulated by E2, but PS-137/pGL3 was not. There are two adjacent GC-rich motifs between -175 and -137, and PS-175Mut/pGL3 containing mutations of these GC-rich motifs did not show E2-dependent repression.

*ER $\alpha$  mediated E2-dependent PROS1 down-regulation:* We also examined the necessity of ER $\alpha$  expression for the *PROS1* repression by E2 in HepG2-derived cells. In HepG2-mock transfected cells, the *PROS1* promoter activity was not down-regulated by E2 treatment, but was in HepG2-ER $\alpha$  cells. A pure ER antagonist, ICI 182,780, blocked this E2-induced *PROS1* repression.

*Transcription factor Sp1, Sp3 and ER $\alpha$  bound to the PROS1 promoter region, and Sp1 was necessary for E2-dependent repression:* Next, we performed the DNA pull-down assay and the electrophoretic mobility shift assay (EMSA) to identify the transcriptional factors interacted with the *PROS1* promoter. In DNA pull-down analyses, we found that Sp1, Sp3 and ER $\alpha$  bound to the oligonucleotide containing the GC-rich motifs of *PROS1* promoter. We also observed the bindings of Sp1 and Sp3 to the same oligonucleotide in EMSA, but could not detected ER $\alpha$  binding. To verify the functions of Sp proteins in the E2-induced *PROS1* repression, we carried out knockdown experiments for Sp1 and Sp3 by RNA interference using specific siRNAs against them. The knockdown of Sp proteins resulted in reduced promoter activities, but E2-dependent repression did not occur by knockdown of Sp1, whereas remaining E2-responsiveness by knockdown of Sp3.

*Sp1/ER $\alpha$  interacted receptor interacting protein 140 (RIP140), and recruited co-repressor-HDAC complex:* We performed the chromatin immunoprecipitation (ChIP) assay and ChIP re-immunoprecipitation (IP) assay to investigate the chromatin states of E2-treated HepG2-ER $\alpha$  cells. In ChIP assays, we observed the bindings of Sp1, Sp3 and ER $\alpha$  to the *PROS1* promoter region containing GC-rich motifs as well as DNA pull-down assay. Sp1 and Sp3 were bound to the GC-rich motifs

in an E2-independent manner, but Sp1 binding was enhanced by E2 treatment. The binding of ER $\alpha$  was completely in an E2-dependent manner. In further analyses of transcription factors interacting with the *PROS1* promoter, we found that co-repressor nuclear receptor co-repressor (NCoR) and silencing mediator of retinoid and thyroid hormone receptors (SMRT) were E2-dependently recruited to the GC-rich motifs. These co-repressors were not known to be recruited by liganded ER $\alpha$ , however, we tried ChIP assays for receptor interacting protein 140 (RIP140) [16] and ligand-dependent co-repressor (LCoR) [17], which are known to interact liganded ER $\alpha$ , and we identified only RIP140 bound to the *PROS1* promoter containing GC-rich motifs. Because RIP140 is also known to interact histone deacetylases (HDACs) [18], we investigated the recruitment of them. We observed class I HDAC; HDAC3 was robustly recruited by E2-treatment, whereas class II HDACs did not affected.

To confirm the interaction of these transcription factors upon the *PROS1* promoter region containing GC-rich motifs, we performed ChIP re-IP assays. The results suggested that Sp1 interacted ER $\alpha$ , and Sp1/ER $\alpha$  recruited NCoR/SMRT-HDAC3 complex via RIP140. A HDAC inhibitor trichostatin A (TSA) blocked E2-dependent repression, so *PROS1* gene repression was mediated HDACs, which induced a hypoacetylated chromatin state and less permissive transcription.

#### Discussion

The decreased level of plasma PS during pregnancy is a well-known risk factor for deep venous thrombosis, but its precise mechanism has not been unknown. In this study, we clarified that PS decrease was induced by 17 $\beta$ -estradiol through transcriptional repression.

Meanwhile, the action of estrogen has been investigated well, which is ligand-dependent modulation of estrogen receptor and regulation of transcription in target gene. Although the positive regulation of target gene expression by estrogen receptor has been reported in various studies, negative regulation of target gene expression has been less understood. Especially, liganded ER had been thought to only regulate the transcription positively. We identified liganded ER has a potential of negative regulation, mediating RIP140 and co-repressor-HDAC complex. RIP140 is known to interact HDACs, whereas HDACs exist sometimes as complex such as NCoR/SMRT-HDAC, so there might be another formation accompanied with ER-RIP140 upon the target gene promoter.

In the down-regulation of protein S gene expression, the interaction of Sp1 and ER $\alpha$  might be important. The Sp1 formed complex with ER $\alpha$  upon the GC-rich motifs of the *PROS1* promoter, and consequently they reinforced the stability of Sp1/ER $\alpha$ -DNA complex. The interacted ER $\alpha$  recruited RIP140, and RIP140 recruited the NCoR/SMRT-HDAC3 complex. The NCoR/SMRT-HDAC3 complex might induce the hypoacetylated histones, in which DNA could coil tightly and limited accessibility of the basal transcription factors and thus repressing the *PROS1* expression.

#### Conclusions

In this study, we have demonstrated that 17 $\beta$ -estradiol down-regulates protein S gene expression via ER $\alpha$ . Liganded ER $\alpha$  interacts basal transcriptional factor Sp1 and recruits RIP140, which associates directly with HDAC3 complex containing NCoR and SMRT. Thus E2-dependent *PROS1* repression results in decreased levels of plasma PS, and this

leads to the risk of deep venous thrombosis during pregnancy or oral contraceptive use.

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## COMPARISON OF REBA HPV-ID<sup>®</sup> WITH DNA CHIP BASED ASSAY FOR HPV GENOTYPING

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#### Introduction

Human papillomaviruses (HPV) have been known to cause human cervical cancer which is the second most common cancer among women worldwide and the most common cancer among women in developing countries [1, 6, 9].

Even though, the HPV prevalence among subgroups of people classified by disease severity varies, overall prevalence of HPV in people with cervical cancer has been reported to reach up to 95 to 100% [9]. So far, more than 100 genotypes of HPV have been known. Among those, certain groups of HPV have been implicated to have high association with cervical cancer, whereas other types have low association [2]. In detail, the most associated geno-types with malignant cervical cancer are 16, 18, 45, 31, 33, 52, 58, and 35. Of these, HPV16 and 18 are mostly related to the malignant cervical cancer [1].

For this reason, HPV genotyping rather than mere detection of HPV has been recommended and thus, the test volume for HPV genotyping has been rapidly increasing. Nowadays, reliable and accurate detection method that able to identify the specific HPV genotypes is required, thus several PCR-based assays have been developed to detect specific genotypes of HPV [8]. Currently, many genotyping tests kits in clinical settings have been commercially available and evaluated to be valuable for clinical diagnosis [3, 8].

This study was set to evaluate a new HPV genotyping kit, REBA HPV-ID<sup>®</sup> from M&D (Wonju, Korea) for its usefulness for genotyping HPV using clinical specimens.

#### Materials and Methods

##### HPV samples and liquid based cytological smears

The clinical specimens were obtained since November of 2008 to July of 2009 using by standard procedure from 356 women in Gangwon province, Korea from Yonsei university Wonju Christian Hospital.

##### Cytological diagnosis

The Liquid-based cytological smears obtained using recommended procedures and clinical diagnoses were interpreted by the pathologists and were classified by 2001 Bethesda System terminology.

##### HPV genotyping

HPV genotyping using REBA HPV-ID<sup>®</sup> (M&D, Wonju, Korea) and MyHPV DNA Chip (Mygene, Seoul, Korea) were carried out according to the manufacturer's recommendation. Both genotyping methods, REBA HPV-ID<sup>®</sup> and MyHPV DNA Chip require the target region (firstly, amplify MY11 and MY9 then, amplify GP5 and GP6) amplification before performing the examination. After amplification of target region by PCR, post PCR steps performed according with manufacturer's

recommendation. Briefly, for REBA HPV-ID<sup>®</sup> assay, PCR products added to the genotype specific probe labeled membrane strips, washed non-specific binds, and then stained with staining solution of PCR product-probe linked region of strips. For MyHPV DNA Chip assay, PCR product loaded to the probe labeled glass chip and then read the result signal using by scanner.

##### Sequencing

Total eight samples were analyzed by sequencing for conformational test. These eight samples have got positive result which appear single HPV genotype infection with REBA HPV-ID<sup>®</sup>, however the result was negative with MyHPV DNA Chip. For sequencing analysis, one-tube nested PCR was performed to amplify the target region (GP5 and GP6) and then, sequencing analysis was performed at Xenotech (Dae-jeon, Korea).

#### Results

##### Clinical diagnosis of HPV

According to the cytological interpretations, 180 specimens (50.56%) were normal, 162 specimens (45.5%) were abnormal, and 14 specimens (3.93%) have not been diagnosed because which were prescribed to perform HPV genotyping directly without cytological diagnoses. Abnormal cytological diagnoses were classified 6 grades. Respectively, 67 (18.82%) ASCUS, 2 (0.56%) ASC-H, 50 (14.04%) LSIL, 30 (8.42%) HSIL, 12 (3.37%) SCC, and 1 (0.28%) C B2C.

##### Genotyping of HPV in abnormal cytology, REBA HPV-ID<sup>®</sup> versus MyHPV DNA chip

The overall positivity of REBA HPV-ID<sup>®</sup> was 80.86% (186) using clinical specimens with abnormal cytological interpretation: the positivity rate of HPV with abnormal cytology was 91.67% (11) in SCC, 96.66% (29) in HSIL, 92% (46) in LSIL, 100% (2) in ASC-H, and 64.17% (43) in ASCUS. When the results from REBA HPV-ID<sup>®</sup> were compared with MyHPV DNA Chip, the overall positivity rate of MyHPV DNA chip was 69.75%, and the positivity rate of HPV with abnormal cytology was 91.67% (11) in SCC, 96.88% (29) in HSIL, 84 % (42) in LSIL, 100% (2) in ASC-H, and 43.28% (29) in ASCUS with MyHPV DNA chip. The positive rate of HPV in ASCUS was significantly lower with MyHPV DNA chip than with REBA HPV-ID<sup>®</sup>. These data shows that REBA HPV-ID<sup>®</sup> has about 30% higher sensitivity than MyHPV DNA chip.

The five most frequently detected HPV sub-types using REBA HPV-ID<sup>®</sup> were HPV16, 53, 58, 56, and 33 with abnormal cytological diagnosis, while using Mygene DNA chip,

HPV subtypes 16, 58, 56, 53, and 18 were most frequently detected.

#### Genotyping of HPV in normal cytology, REBA HPV-ID<sup>®</sup> versus MyHPV DNA chip

On the other hand, the rate of HPV positive was 64.44% (116) in REBA HPV-ID<sup>®</sup> and 34.44% (62) in MyHPV DNA chip based assay with normal cytology. The data shows REBA HPV-ID<sup>®</sup> has about 2 fold higher positivity than DNA chip with normal cytological diagnosis. In this study, The five most remarkable HPV sub-types detected were HPV16, 53, 58, 18, and 56 with normal cytological diagnosis using REBA HPV-ID<sup>®</sup> and HPV16, 52, 18, 53, and 58 using MyHPV DNA chip. These data suggest that REBA HPV-ID<sup>®</sup> has higher specificity than MyHPV DNA chip.

#### Sequencing analysis with specimens that have different results

To confirm the accuracy and reliability of two methods, sequencing analysis was performed. Before the sequencing analysis, sorting of suitable specimens was required. Total seven specimens, which have negative results with MyHPV DNA Chip, have a strong signal of PCR and have single genotype infection result (one-band) with REBA HPV-ID<sup>®</sup>, were selected. GP5, GP6 region was amplified and sequenced. Five out of seven samples sequenced clearly to interpret data and these results were corrected with REBA HPV-ID<sup>®</sup> because these samples had negative results with MyHPV DNA Chip. The rest two samples could not be interpreted by sequencing analysis. They would be mixed infection which infected with more than two genotypes of HPV or non-HPV infection (negative). Therefore, these data suggest that REBA HPV-ID<sup>®</sup> has higher sensitivity (72.42%) and accuracy than MyHPV DNA Chip (28.57%).

#### Discussion

In this study, we evaluated the performance of the REBA HPV-ID<sup>®</sup> system which based on reverse blot hybridization assay compare with the results of DNA chip assay and cytological interpretation with clinical specimens.

Recently available HPV genotyping kit REBA HPV-ID<sup>®</sup> has several advantages over a DNA chip based HPV genotyping method. First of all, REBA does not require expensive equipments such as scanner. Second, it is more suitable for the laboratory with large volume of tests by saving labor and reducing turn-around time. The total time required for the REBA HPV-ID<sup>®</sup> was from PCR to genotyping analysis was about 4 hours. In brief, it can be more economical and time consuming.

According to our finding, the positivity of the REBA HPV-ID<sup>®</sup> was higher (80.86%) than other DNA chip based method (69.75%) in abnormal cytological samples and also positivity of REBA HPV-ID<sup>®</sup> (64.44%) was higher than MyHPV DNA chip (34.44%) in normal cytological samples. Thus, it seems that REBA HPV-ID<sup>®</sup> would provide more sensitive and specific detection rate for samples with abnormal and normal cytology.

To confirm the reliability of REBA HPV-ID<sup>®</sup>, Sequencing analysis was performed. Sequencing results show that REBA HPV-ID<sup>®</sup> was more reliable and higher sensitivity.

#### Conclusions

Sequencing analysis has a limitation that is not a golden standard method as a confirmative test because if some patients who infected with more than 2 HPV genotypes, the results could not be interpreted clearly. Therefore, other kinds of confirmative tests are required for accurate HPV genotype detection and need to further evaluation.

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## Morphometric and FISH Analyses of Adenocarcinoma and Mesothelial Cells Using Liquid-Based Cytology

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#### Introduction

Cytological diagnosis of malignant effusions is often difficult mainly because of the presence of reactive mesothelial cells. The evaluation of cellular morphology with conventional Papanicolaou or Giemsa stains tend to be more or less subjective as shown by the interobserver and even the intraobserver variabilities. Immunocytochemistry has been applied to aid the differentiation of adenocarcinoma and reactive mesothelial cells, but is not very useful largely because of the limitation of the specimen. LBC is superior to conventional cytology in that it provides smear slides with a uniform cell density and a distribution that enables a precise cytological examination. Moreover, one can preserve cells in the LBC solution for a long period of time and always utilize it for a variety of purposes, such as immunocytochemistry and molecular analysis. To establish a more objective diagnostic system, we performed morphometric and FISH analyses on adenocarcinoma and mesothelial cells, using a liquid-based cytology method, and evaluated its usefulness.

#### Materials and Methods

Fourty five tumors of alimentary tract, 20 gastric adenocarcinomas and 25 colonic adenocarcinomas, were studied. Cells were harvested from the surface of tumor and serosa of resected fresh specimen, suspended in the solution for fixation and preservation (Liqui-PREP™ Preservative Solution), and processed for Papanicolaou staining and FISH. Digital images were taken from Papanicolaou-stained slides for the following measurements. The nuclear area and perimeter of 100 cells per each case were measured using the software ImageJ (NIH), and the mean nuclear area (NA), the mean nuclear perimeter (NP), the mean length of nuclear longer axis (LLA) and their standard deviations (SNA, SNP, SLLA) were calculated. The shape factor (SHF) was obtained by the following formula:  $(4 \times \pi \times \text{area}) / \text{perimeter}^2$ . SHF is dimensionless and its value is equal to 1.0 in circles and <1.0 in spheroid nuclei (Figure 1). Standard deviations of the shape factor (SSHF) were also calculated. FISH analysis was performed to detect chromosome 8 polysomy and c-myc amplification.

#### Results

The nuclear area (NA) was smaller in gastric adenocarcinoma cells than in colonic adenocarcinoma cells and mesothelial cells, although the differences were not statistically significant. Results of the standard deviation of nuclear area (SNA) demonstrated that adenocarcinoma cells had more variable-sized nuclei than mesothelial cells (\*\*P<0.01) (Figure 2).

Results of the nuclear perimeter (NP) showed a similar tendency to NA. As for the standard deviation of nuclear perimeter (SNP), colonic adenocarcinoma cells showed the highest value, followed by gastric adenocarcinoma cells, and mesothelial cells showed the lowest value (\*\*P<0.01) (Figure 2).

As for the length of nuclear longer axis (LLA), colonic adenocarcinoma cells had longer nuclei (\*P<0.05, \*\*P<0.01) than gastric adenocarcinoma cells or mesothelial cells without a significant difference between the latter two. Results of the standard deviation of length of nuclear longer axis (SLLA) showed a similar tendency to SNP (\*\*P<0.01) (Figure 2).

As for the shape factor (SHF), mesothelial cells had the most rounded nuclei, followed by gastric adenocarcinoma cells, and nuclei of colonic adenocarcinoma cells were the least rounded (\*\*P<0.01). Results of the standard deviation of shape factor (SSHF) showed a similar tendency to SNP and SLLA (\*\*P<0.01) (Figure 2).

Adenocarcinoma cells of both stomach and colon in all cases showed significant percentages of chromosome 8 polysomy and c-myc amplification, whereas mesothelial cells in all cases showed normal cell pattern.

#### Discussion

Morphometric analysis revealed that gastric adenocarcinoma cells were less atypical and more uniform than colonic adenocarcinoma cells and had even smaller nuclei than mesothelial cells. One should always take these facts into consideration on cytological examination, since gastric and colonic adenocarcinoma cells are definitely the most frequently encountered atypical cells in intraoperative peritoneal washing cytology, although cellular atypia should be more integrally assessed using other indices. By FISH analysis using LBC specimens, quantitation of positive signals was more easily performed than with histology sections, resulting in detection of chromosome 8 polysomy and c-myc amplification in significant percentages of both gastric and colonic adenocarcinoma cells, whereas all the mesothelial cells showed the normal pattern.

#### Conclusions

Morphometric and FISH analyses, together with conventional stains and immunocytochemistry, using LBC method can be used as ancillary techniques in differentiation of adenocarcinoma and mesothelial cells.

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Figure 1. A: The shape factor (SHF) is dimensionless and its value is equal to 1.0 in circles and <1.0 in spheroid nuclei. B: The shape factor (SHF) of this picture shows 0.743, the nuclear area (NA) is 84.065  $\mu^2$ , the nuclear perimeter (NP) is 37.700  $\mu$  m, and the length of nuclear longer axis (LLA) is 14.183  $\mu$  m.

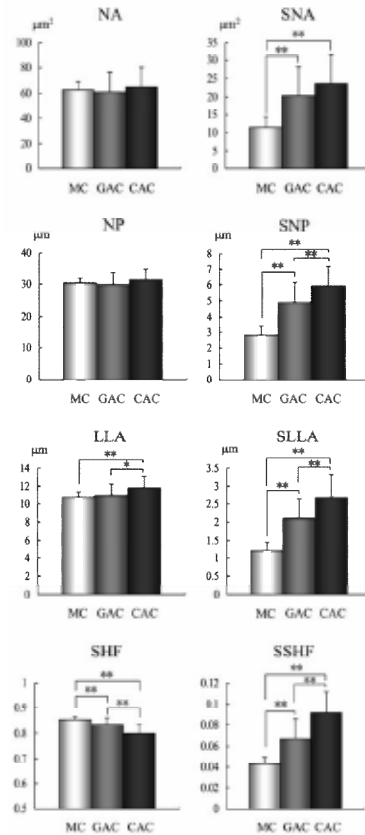


Figure 2. Results of morphometric analysis of mesothelial cells and adenocarcinoma cells on LBC slides. MC: mesothelial cells, GAC: gastric adenocarcinoma cells, CAC: colonic adenocarcinoma cells.

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## MOLECULAR MECHANISM OF CERAMIDE KINASE GENE REPRESSION ON ATRA-INDUCED NEURONAL DIFFERENTIATION

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### Introduction

Ceramide kinase (CERK) is the enzyme that phosphorylated ceramide to ceramide 1-phosphate (C1P).

Previous studies suggested that ceramide kinase (CERK) and its product, ceramide 1-phosphate (C1P) have been implicated in various cellular function including cellular proliferation, survival, mast cell degranulation, and phagocytosis.

However, the regulatory mechanism of CERK gene expression remains to be determined.

In the current study, we examined the regulatory mechanism of CERK gene expression during all-*trans* retinoic acid (ATRA)-induced neuronal differentiation of a human neuroblastoma cell line, SH-SY5Y cells.

### Materials and Methods

**Cell line:** A human neuroblastoma cell line, SH-SY5Y, was cultured in Dulbecco's Eagle's medium supplemented with 10% fetal bovine serum.

**Establishment of CERK stable transfectants:** Human CERK cDNA was the kind gift of Dr. Kohama T. (Daiichi-Sankyo Co., Ltd., Tokyo, Japan). hCERK cDNA was inserted into pcDNA3.1(+) expression vector (Invitrogen, California, USA). To establish the stable transfectants of CERK, DNA transfection was performed by the calcium precipitation method. A subclone (sc 17) showing the highest CERK protein expression was used for further experiments.

**Cell proliferation and cell death:** Cell proliferation was determined in triplicate with WST-1 assay kit (Roche Applied Science, Mannheim, Germany). Cell death was determined with LDH-cytotoxic test (Wako Pure Chemical Industry, Osaka, Japan).

**CERK enzyme activity:** CERK activity was determined as described previously [1] with some modification.

**Western blotting:** Western blotting was performed using ECL plus western blotting system (Amersham Pharmacia Biotech, Buckinghamshire, UK).

**Quantitative RT-PCR:** Quantitative RT-PCR was performed as described previously [2]. The primers were as follows: CERK sense 5'-AGTCCACCACACAGCAC-3', antisense 5'-GAGGAAGGTCCTTTAAACCTG-3', Sphingosine kinase 1 (SPHK1) sense 5'-TCCTGGCACTGCTGCATC-3', antisense 5'-TAACCATCAATTCCTCCATCCAC-3', and GAPDH sense 5'-CAGGAGCGAGATCCCTCCAA-3', antisense 5'-CCCTGCAAAATGAGCCC-3'.

**Cloning of the 5'-promoter region of human CERK:** Human CERK 5'-promoter region was prepared using a PCR-based method. Using suitable restriction enzymes, this promoter region was inserted into pGL3 Basic vector (Promega, Madison, USA). The deletion mutants were

prepared using Exonuclease III digestion, PCR-based cloning, and restriction enzymes digestion method. To introduce the mutation to the RARE like motif, overlap extension PCR method was performed.

**Luciferase assay:** SH-SY5Y cells were plated at a density of 5x10<sup>5</sup> cells/ml in 35mm dishes. After 2 days of incubation, 0.5 $\mu$ g of promoter construct and 2 $\mu$ g of  $\beta$ -galactosidase expression vector were co-transfected into cells using lipofectin reagent (Invitrogen) according to the manufacturer's protocol. Luciferase activity and  $\beta$ -galactosidase activity were measured according to the method described previously [3].

**Electrophoresis mobility shift assay (EMSA):** EMSA was performed as described previously [4]. One  $\mu$ g of nuclear extract and 600 fmol of biotin-labeled probe were incubated at room temperature for 40 min. Cold probe was used for the competition experiment (x20 excess). For the supershift experiments, anti-RAR $\alpha$  antibody, anti-RXR $\alpha$  antibody, and anti-COUP-TF1 antibody (Santa Cruz) were used.

**DNA pull-down assay:** Nuclear extract, biotin-labeled double strand probe and Poly dI-dC were incubated in DNAP buffer for 30min on ice. Following the incubation, Dynabeads M-280 streptavidin (Invitrogen Dynal AS, Oslo, Norway) were added and mixed with rotator at 4 $^{\circ}$ C for 30 min. Prior to this step, the beads were washed in DNAP buffer for 10 min with rotator at three times. After washing, dissolved in SDS sample buffer and heated at 95 $^{\circ}$ C for 5 min. Western blotting was performed using anti-RAR $\alpha$ , anti-RXR $\alpha$ , and anti-COUP-TF1 antibody.

**Chromatin immunoprecipitation assay (ChIP assay):** ChIP assay was performed as described previously [4]. For immunoprecipitation, normal IgG, anti-RAR $\alpha$ , anti-RXR $\alpha$ , anti-COUP-TF1, anti-N-CoR, anti-SMRT, and anti-HDAC3 antibody (Santa Cruz) were added to the lysate. The CERK promoter region was amplified by PCR using the following primer set. Sense: 5'-GTCCCTCCGCGGTCCCC-3', antisense: 5'-GCTTCACCCACAGCAGGGATT-3'.

**Transient transfection followed by co-immunoprecipitation:** The plasmids of RAR $\alpha$ , RXR $\alpha$ , and COUP-TF1 were the gift of Dr. W. H. Miller (McGill University, Quebec, Canada), Dr. R.M. Evans (The Salk Institute for Biological Studies, CA, USA), and Dr. G. Salbert (Universite de Rennes I, Rennes, France), respectively. After transfection with respective plasmid DNA using lipofectin reagent (Invitrogen), SH-SY5Y cells were treated with either ATRA or methoprene acid, a specific agonist of RXR $\alpha$ , for 24 h. Co-Immunoprecipitation assay was performed by described previously [5] with some modification.

### Results

ATRA reduced CERK enzyme activity, CERK protein, and mRNA expression whereas another sphingolipid metabolite

enzyme, SPHK1 that had similar function to CERK, did not show significant changes with ATRA.

To examine transcriptional regulation of ATRA-induced CERK gene repression, promoter analysis was performed.

ATRA reduced CERK promoter activity, and this reduction was mainly because of the region between -40 and -28 bp from the first exon. In this region, the tandem retinoic acid responsive element (RARE) like motifs were identified with one base space. Introduction of mutation into these RARE like motifs, erased ATRA-induced inhibition of CERK promoter activity.

To determine which transcription factor was bound in these RARE like motifs, EMSA, DNA-pull down assay, and ChIP assay were performed.

These experiments revealed that ATRA induced binding of retinoic acid receptor  $\alpha$  (RAR $\alpha$ ), retinoid X receptor  $\alpha$  (RXR $\alpha$ ), Chicken ovalbumin upstream promoter transcription factor 1 (COUP-TF1) to these RARE like motifs. Moreover, ChIP assay showed that HDAC3, N-CoR, and SMRT were recruited to these RARE like motifs.

Transient expression of RAR $\alpha$ , RXR $\alpha$ , and COUP-TF1 and siRNA transfection of these genes concluded that COUP-TF1 inhibited CERK mRNA.

Another ATRA-induced differentiation model, human acute myeloblastic leukemia cell line, HL60 cells, showed some increase of CERK mRNA with ATRA. Interestingly, ATRA induced binding of RAR $\alpha$  and RXR $\alpha$  to these RARE like motifs, but COUP-TF1 didn't.

These results suggested that the relative ratio of RAR/RXR and COUP-TF1/RXR or COUP-TF1/RAR might determine the level of CERK transcription in different cell lines.

To examine a function of CERK on cell proliferation, cell death, and neuronal differentiation of SH-SY5Y cells, we established stable transfectants of CERK. CERK over-expression counteracted ATRA-induced inhibition of cell proliferation and cell death under the serum starvation. On the contrary, siRNA of CERK enhanced the effect of ATRA on cell growth and cell death.

Furthermore, to examine the effect of neuronal differentiation, ATRA-induced neurite extension and growth associated protein-43 (GAP-43) protein expression, which was main component of growth cone, was examined. CERK over-expression inhibited ATRA-induced neuronal differentiation. On the contrary, siRNA of CERK enhanced neuronal differentiation.

These results revealed that CERK promoted cell proliferation and cell survival, and had an inhibitory effect against ATRA-induced neuronal differentiation.

#### Discussion

Recent analysis revealed that sphingolipid and their metabolic enzymes were involved in cellular signalling, cell survival, and cell death. CERK and its product, C1P have also been suggested to play an important role of cell survival and inflammation [6, 7]. Intriguingly, CERK is mainly distributed in brain, especially, highly expressed in cerebellum [8].

Our results of stable transfectants of CERK and siRNA of CERK not only confirmed previous results but also revealed CERK as the unique modulator of neuronal cell function in SH-SY5Y cells. It is very interesting that further analysis of elucidating the function of CERK in central nervous system.

We recently reported that SPHK1 gene expression was increased during glial-cell line derived neurotrophic factor (GDNF) induced neuronal differentiation of human

neuroblastoma cell line, TGW cells [9]. But in ATRA-induced neuronal differentiation of SH-SY5Y cells, SPHK1 expression didn't show any change. This result suggested that CERK and SPHK1 play non-overlapping roles in neuronal differentiation.

Vitamin A and its major metabolite, ATRA, are essential for network formation during early brain development [10]. COUP-TF1 involvement in modulating ATRA-induced differentiation has been reported in multiple cell types [11, 12]. Our results suggested that COUP-TF1 modulated ATRA-induced CERK expression, and the possible role of CERK in the early physiological process of normal neuronal differentiation.

#### Conclusion

CERK is antagonistic to ATRA-induced neuronal differentiation and that a tandem RARE like motifs located in the 5'-promoter region was responsible for ATRA responsiveness, and found that COUP-TF1 induced with ATRA treatment was responsible for CERK gene repression.

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Physical Therapy (13:30 ~16:00 Lecture Room 3)

Chaired by the academic adviser of each presenter

## DEVELOPMENT OF AN INSTRUMENT FOR MEASURING PATELLAR MOBILITY AND ITS CLINICAL APPLICATION

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### Introduction

Passive patellar mobility was believed to be one of the important forms of assessment for patient with patellofemoral pain (PFP) and the decreased range of motion (ROM) at the knee joint. Moreover, it shows the laxity of the knee joint which could also contribute to sports knee injuries.

However, there is no methods to assess the quantitative measurement of patellar mobility in clinical situation. Given the need for objective measurements of patellofemoral (PF) joint impairments, we have developed a clinical device (patellofemoral joint arthrometer [PFA]) to quantify medio-lateral patellar displacement.

The purposes of this research were 1) to assess the validity of prototype PFA, 2) to apply PFA to the person with PF and to compare with the healthy subjects, 3) to investigate the relationship of patellar mobility and knee ROM after total knee arthroplasty (TKA).

### Materials and Methods

#### Study 1<sup>1)</sup>

**Subjects:** A total of 26 individuals (24 females and 2 males) participated, and 14 were asymptomatic with PFP. The average  $\pm$  SD age, height and body mass of the participants was  $27 \pm 4$  years,  $164 \pm 5$  cm, and  $59 \pm 8$  kg, respectively. Twelve of the participants had a current history of patellofemoral pain at the time of testing, while 14 were asymptomatic.

**Methods:** A custom designed prototype PFA was used to assess the amount of lateral patellar displacement. PFA was secured to the femoral condyle, and the femur was refer to this measurement. The adjustable arm allowed the medial border of the patella to be localized in the frontal plane with respect to the fixed ruler. Subjects were measure the displacement of lateral patella between quadriceps relaxed and isometric contracted by PFA. Following assessment of lateral patellar displacement using the PFA, participants underwent MRI of the patellofemoral joint. The same procedures of using PFA was performed on using MRI. Bisect offset index and patellar tilt angle was measured the true lateral patellar displacement. The comparison of these patellar mobilities using both methods was performed as the validity study.

#### Study 2<sup>2)</sup>

**Subjects:** The study comprised 22 females with PFP (PFP group) and 22 females who had no knee pain (control group) who were matched to the subjects with PFP based on age, height, and body mass index.

**Methods:** Patellar mobility was measured objectively using PFA. Measurements of lateral and medial patellar displacement, patellar mobility balance (lateral minus medial patellar displacement), lateral patellar mobility index (LPMI: lateral patellar displacement/ patellar width), and medial

patellar mobility index (MPMI: medial patellar displacement/ patellar width) were used.



Figure 1. Patellofemoral arthrometer



Figure 2. Initial position of the patellar mobility (set 0 mm of the digital caliper)

The PFA was clamped to the femoral condyles aligned parallel to the table. The digital caliper was positioned at an angle of 90 degrees to the line between the center of the patella and the ASIS using the plane adjuster. Before each subject was measured, the amount of force (approximately 80N) to be used to move the patella for testing was practiced using a hand-held dynamometer, MicroFET2 (Hoggan Health Industries Inc., West Jordan, UT) to enable the examiner "to feel" what it was like to apply an 80N force. For testing, relaxation of the musculature was confirmed by palpation of the quadriceps and passively moving the patella in the lateral and medial directions.<sup>17</sup> To measure lateral patellar mobility, the lateral border of the patella was palpated and located with a laser using the adjustable laser module arm, and the digital caliper was zeroed, setting this point as the initial position. Lateral displacement of the patella was then achieved by manually pushing the patella laterally (applying approximately 80N of force), at which point the lateral border of the patella was again

located by sliding the laser module arm on the caliper and reading the measurement. Medial passive patellar mobility was assessed in the same manner except for the force application being applying toward the medial direction. All of these procedures for passive patellar mobility were performed by the same tester for all subjects.

**Study 3<sup>3)</sup>**

Subjects: Fifty-one patients [osteoarthritis (OA) group: 34 knees; rheumatoid arthritis (RA) group: 22 knees] were examined after TKA.

Methods: Patellar mobility was measured preoperatively and 1, 2, and 6 months postoperatively using a patellofemoral arthrometer (PFA) fixed to the femoral condyles to measure medial and lateral patella displacement at 30 degrees of knee joint. Knee joint ROM was also measured in each of these 4 sessions.

All participants in these studies were approved by each Ethics Committee of the University of Southern California and the school of Health Sciences, Nagoya University.

**Data analysis:** Patellar mobility was measured three times, and the averages of the displacements were used in these studies.

For the validity study was used intra-class correlation (ICC2,k), and t test was performed to compare the displacements between PFP group and control group in study 2. For the analysis of the relationship between patellar mobility and ROM in the study 3 were used the Pearson correlation. The differences between each session was using ANOVA and Tukey's procedure. Significance was set at  $P < 0.05$ .

**Results**

**Study 1:** The ICC assessing the level agreement between the MRI and PFA measures of lateral patellar displacement was good (0.86). Excellent intratester (ICCs of 0.96 and 0.97) and intertester reliability (ICC 0.92) was demonstrated.

**Study 2:** Lateral and medial patellar mobility values were not significantly different between the individuals in the PFP and control groups. When normal patellar mobility was arbitrarily defined as the average  $\pm$  2SD based on the data from the control group, normal lateral patellar displacement was within a range of 7.2 to 17.6 mm and normal medial patellar displacement was within a range of 6.8 to 14.0 mm.

**Study 3:** In the OA group, lateral patellar mobility was positively correlated with knee flexion angle, while there was no correlation between medial patellar mobility and knee flexion angle. Knee joint extension was not correlated with either lateral or medial patellar mobility. In the RA group, both lateral and medial patellar mobility were positively correlated with knee joint flexion/extension 1 and 2 months after TKA.

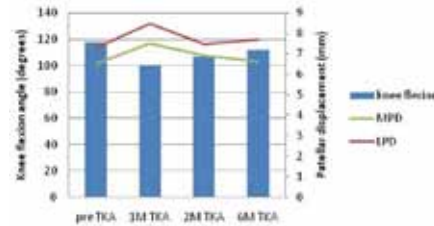
Although, knee ROM were significantly improved from 1 month to 6 months after TKA, medial and lateral patellar mobility were not improved.

**Discussion**

From the study 1, the validity of the prototype PFA compared to MRI method was acceptable range in the clinical application. This prototype was made of polyethylene for using in side of MR imaging.

Study 2 was introduced to PFA to apply the clinical evaluation. PFA was adjusted the patellar mobility using equipped the adjustable lever, lever joints and laser. Based on

an arbitrarily defined normal range of values calculated using the mean  $\pm$  2SD of the patellar displacements measured for the individuals in the control group, we note that the distribution of the data points are very similar between the 2 groups, with only few subjects with PFP falling outside what we considered a normal range of scores. From the results of this study, abnormal patellar mobility might not be a factor in female adults with mild symptoms of PFP.



**Figure 3. Changes of knee flexion angle and patellar mobility in OA group**

In the study 3, although patellar mobility was shown to be related to knee ROM after TKA, there were differences in the relationship between OA and RA groups. In both groups, lateral patellar mobility was positively related to knee flexion angle 1 and 2 months after TKA. In the OA group, knee extension was not associated with patellar mobility, while lateral and medial patellar mobility were related to knee ROM (flexion and extension) after TKA in the RA group. Although these features should be taken into consideration in planning of physical therapy treatment regimens to improve patellar mobility after TKA, the relations identified in the present study represent only cross-sectional findings. However, our findings demonstrated that medial and lateral patellar mobility had no sufficient longitudinal relationship with knee ROM after TKA.

**Conclusions**

We have developed PFA to assess the patellar mobility quantitatively, and we applied it to patients with PFP and after TKA. The patellar mobility might not be a factor in this research subjects with PFP (mild symptom). The knee ROM after TKA could not be related to medial and lateral patellar displacement. This study would be a framework for a future clinical trial on patellar mobility in patient with knee disorder.

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**CD105 POSITIVE MESENCHYMAL STEM CELLS FROM MOUSE ES CELLS SHOW HIGH POTENTIAL FOR DIFFERENTIATION**

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**Introduction**

Mesenchymal stem cells (MSCs) have a potential to differentiate into any mesenchymal cell type such as osteocytes, chondrocytes, adipocytes and muscular cells. Recently, adipose tissues are indicated as a useful and rich source of adipose tissue-derived mesenchymal stem cells (ADSCs). Although ADSCs have therapeutic efficiency in repairing damaged mesenchymal tissues, isolation and purification of ADSCs from adult adipose tissue still requires complicated and troublesome processes[1,2]. Mouse embryonic stem cells (ES cells) are pluripotent, and their induction to adipogenesis has been well described[3,4]. We initially supposed that MSCs would differentiate through the process of adipogenesis from undifferentiated ES cells (Figure 1). The aim of this study was to establish a method for the induction and collection of MSCs using a typical cell surface marker CD105 through adipogenesis from ES cells without genetic manipulation.

**Materials and Methods**

ES cells (G4-2; kind gifts from Dr. Niwa) carrying the enhanced green fluorescent protein —EGFP— gene under the control of cytomegalovirus/chicken  $\beta$ -actin promoter were expanded their population and embryoid bodies (EBs) were formed in hanging drops. EBs were cultured in a retinoic acid containing medium. After washing, they were settled on culture dishes and maintained with adipogenesis medium (insulin / triiodo-thyronine). After the increase of CD105+ cells, we isolated and sorted them by a magnetic cell sorter (MACS; Miltenyi). Purification of CD105+ cells using MACS was confirmed by flow cytometry (FACS). CD105+ cells were then differentiated into mesenchymal cells such as adipocytes, osteocytes, chondrocytes, and skeletal muscles. Differentiation was examined by oil red O, alizarin red, alcian blue, and M-cadherin and/or skeletal muscle myosin heavy-chain (MHC) immuno-staining, respectively. Expression of each cell type-specific mRNA was analyzed by RT-PCR. Obtained CD105+ cells were then injected into tibialis anterior muscles of immunodeficient (SCID) mice to demonstrate their potential for differentiation in vivo.

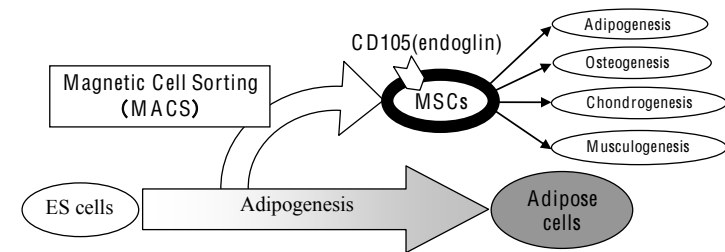
**Results**

*Appearance and Sorting of CD105+ Cells*

After 10 days of induction to adipogenesis, small, round CD105+ cells appeared, began to increase, and reached their maximum population on day 14. After that adipocytes then increased and CD105+ cells alternately decreased. Since double staining with CD105 and BODIPY demonstrated that adipocytes did not express CD105, sorting by MACS was usually carried out on day 14. The efficiency of sorting by MACS was confirmed by FACS analysis, and more than 91% of cells were CD105+ after MACS separation. Sorted CD105+ cells were uniformly bipolar in a shape similar to that of the ADSCs previously reported.

*Differentiation Potential of CD105+ cells to Mesenchymal lineages in vitro*

CD105+ cells showed a high potential for differentiation into mesenchymal cells in vitro, when both CD105+ and CD105- cells were induced to mesenchymal cells and compared with each other. After induction of adipogenesis, almost all CD105+ cells differentiated into adipocytes. CD105- cells, however, did not produce large Oil Red O-positive oil droplets. CD105+ cells turned Alizarin Red-positive after induction of osteogenesis. The CD105- cells did not survive under the condition of osteogenesis and disappeared. Under the pellet culture for chondrogenesis, the CD105+ cells formed small ball like-aggregations. When a cryo-section of the aggregations was stained with Alcian blue, the matrix surrounding the chondrocytes turned blue. The matrix produced by CD105+ cells also expressed collagen type II immunoreactivity, while the CD105- cells did not form cell aggregations and were not stained with either Alcian blue or anti-collagen type II antibody. When CD105+ cells differentiated into skeletal muscle cells, they became thin and fibrous, and began to express M-cadherin. They ran in the same direction and distributed parallel to each other. They finally constructed sarcomeres exhibiting bands with myosin and actin filaments regularly arrayed. A few CD105- cells expressed M-cadherin immunoreactivity, but they did not form myotubes under the same condition.



**Fig. 1 Schematic view of the induction and isolation of MSCs from ES cells**

Potential of CD105+ cells for differentiation into mesenchymal cells was confirmed as well by the expression of their specific mRNAs detected by RT-PCR.

After the induction of adipocytes and the formation of lipid droplets, CD105+ cells expressed *PPAR-γ* and *LPL* mRNAs. Following the formation of osteogenic cells, they displayed both *Runtx-2* and *Osterix* mRNAs. The small ball-like structures observed after the induction of chondrogenesis expressed *Collagen2* and *agglycan* mRNAs. Skeletal muscle cells differentiating from CD105+ cells expressed *Myogenin*, *M-cadherin*, *MyoD* and *Myf5* mRNAs.

#### Differentiation Potential in vivo; Transplantation to Immunodeficient (SCID) Mice

Potential for differentiation into mesenchymal cells in vivo was examined by transplantation to SCID mice. CD105+ cells were transplanted into injured tibialis anterior muscles 24 hours after clamping. Transplanted cells located at the injected area were clearly distinguished from host cells by their EGFP immunoreactivity. After 1 week of transplantation, EGFP-positive cells showed M-cadherin immunoreactivity. Some of them express MHC immunoreactivity. Their nuclei were located at the center of the cells and occasionally appeared as multinuclear cells in the cross section. After 2 weeks of transplantation, EGFP and MHC-double positive cells increased and became large enough to involve several nuclei in the cross section. From the HE staining, many blood vessels invaded the colony of transplanted cells. Flk1-positive cells indicated angiogenesis.

#### Discussion

##### Expression and Specificity of CD105

CD105, also known as endoglin, is a homodimeric type transmembrane glycoprotein that binds to TGF-β1 and TGF-β3 to form a receptor complex comprised of TGF-β receptors I and II. TGF signaling plays important roles in cell growth, differentiation and migration, and is also known as a functional marker for the isolation of mesenchymal stem cells including ADSCs. Almost all reports describing mesenchymal stem cells have demonstrated the expression of CD105 on the cell surface, further gene expression profiles of mesenchymal stem cells have involved CD105 [2,5]. After the completion of adipogenesis, mature adipocytes did not express CD105. This indicates that only stem cells or progenitor cells express CD105, whereas mature mesenchymal cells themselves lose the expression. Therefore, CD105 was confirmed as a useful marker to separate and collect mesenchymal cells in our system. The isolation efficiency rate by MACS was more than 91%. This was further enhanced using a technique of adhesion separation after sorting by MACS.

##### Potential of Differentiation into Mesenchymal Cells in vitro and in vivo Indicates MSCs

CD105+ cells demonstrated a high potential for differentiation into mesenchymal-type cells, i.e., adipocytes, chondrocytes, osteogenic cells and skeletal muscles in vitro and in vivo. Their differentiation potential indicates that CD105+ cells were mesenchymal stem cells (MSCs). Our MSCs were easily differentiated into skeletal muscles, though ADSCs have been described as hardly differentiating into muscles [6]. In general, the requirement of the provision for specific ADSCs does not in fact involve the potential for skeletal muscle differentiation [2]. The prominent potential of our MSCs to differentiate into skeletal muscle probably depends on the medium containing insulin and T3 for

adipogenesis before sorting by MACS. Skeletal muscle cells are sensitive to insulin and the IGF family as growth factors. IGF-II promotes myogenesis through Rho-ROCK activity and induces myoD1 [7]. The receptors of insulin and/or IGFs may be highly expressed before induction of myogenesis in our MSCs. Culture conditions generating our MSCs showed advantages over myogenesis in our method.

Transplantation to the injured tibialis anterior muscles differentiated MSCs into skeletal muscles in vivo. This suggests that the fate of MSCs ultimately depends on their micro environments as already described [2]. Induction factors from host tissues surrounding MSCs regulate their differentiation into a certain mesenchymal cell type. Further studies of the molecular mechanisms of differentiation into specific mesenchymal cell type by MSCs should advance the MSC cell therapies.

#### Conclusions

We developed MSCs with a high potential for differentiation into mesenchymal cell lineages, including skeletal muscles in vitro from ES cells using CD105 as a cell surface marker without gene manipulation. Depending on their surrounding micro-environments in vivo, MSCs differentiated into skeletal muscles of injured tibialis anterior muscles. All properties of our MSCs proved very beneficial to cell-derived regeneration and therapeutic usage.

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## EFFECTS OF FORCED IMPAIRED FORELIMB USE ON CENTRAL NERVOUS SYSTEM AFTER SMALL INTERNAL CAPSULE HEMORRHAGE IN RATS

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#### Introduction

Forced impaired forelimb use (FLU) is known as an effective method to promote functional recovery of the affected forelimb after hemiplegic stroke (e.g., constraint-induced movement therapy) [1,2]. However, the detailed mechanism of FLU effect on the brain was still unclear. In this study, we investigated whether FLU induces functional recovery after small intracerebral hemorrhage (ICH) and how the dominant use induces improvement of motor functions.

#### Materials and Methods

**Experimental setup:** Adult male Wistar rats (250-300g) were housed at a 12 h light/dark cycle with food and water ad libitum. Rats were randomly assigned to four groups: ICH (n=8), ICH-FLU (n=9), Sham (n=5) and Sham-FLU (forced-preferred limb use: n=6). All experimental procedures were performed in accordance with the animal care guidelines of the Nagoya University. Figure 1 shows a timeline of the performed experiments.

**Intracerebral hemorrhage:** In the present study, we used internal capsule hemorrhage model [3]. Under deep anesthesia, 1.4 μl Collagenase (15 Units/ml, Type IV; Sigma-Aldrich, St. Louis, MO, USA) were injected into the internal capsule contralateral to the preferred forepaw. Figure 2 shows a typical histological appearance of hemorrhage.

**Forced-impaired limb use:** FLU-treated animals were fitted with a 1-sleeve plaster cast 24hrs after ICH surgery. The upper torso and the unimpaired/unpreferred forelimb were wrapped in soft felt and Plaster of Paris strips. Rats were forced to completely rely on either their impaired/preferred forelimb for 1 week.

**Behavioral assessment:** After restraint, rats were assessed various forelimb motor functions, such as skilled reaching (assessed by single pellet reaching test), coordinated forelimb stepping (by ladder test), spontaneous use of the impaired forepaw (by cylinder test), and tactile-evoked forelimb placing (contact placing response). Figure 3 shows the appearances of these tests. Assessment was carried out at postoperative day 10-12 and 26-28. Before ICH, single pellet reaching test and ladder test were pre-trained and determined baseline performance.

**Lesion volume:** After the completion of behavioral testing, all animals were deeply anesthetized and perfused transcardially with 0.9% saline followed by 4% phosphate-buffered paraformaldehyde (pH 7.4). The brains were removed and postfixed, and then 40 μm thick coronal sections were obtained using a cryostat. These sections were processed with hematoxyline-eosin staining and volume of lesion was determined.

**Retrograde labeling of corticospinal tract:** An additional group of rats (ICH; n=6, ICH-FLU; n=6, Sham; n=9) was used for quantification of the remaining corticospinal tract. After same FLU treatment, animals were anesthetized and their spinal cords were exposed at the C6-C8 level using laminectomy. 4% fluorogold (FG; Biotium, Hayward, CA) was injected into the spinal cord with a glass micropipette.

Each animal had four injections of 250 nl of FG spaced at 0.5 mm, along the spinal cord (0.75 mm lateral from the midline and 1.2 mm below the spinal cord surface). Seven days after injection, animals were perfused and processed for cryosectioning. Series of 30 μm brain cross sections (with 180 μm gaps) were used for quantification of labeled cells.

**Real-time PCR:** Separate cohorts of animals exposed to the same FLU treatment were used for quantitative transcription polymerase reaction (PCR) analysis (ICH; n=6, ICH-FLU; n=6, Sham; n=6, Sham-FLU; n=6). Sensorimotor cortex forelimb region harvested at the end of the FLU period was analyzed by real-time PCR of brain-derived neurotrophic factor (BDNF) and growth-associated protein 43 (GAP43).

**Statistical analyses:** One-way ANOVA followed by a Tukey post hoc test was used for statistical analyses of behavioral tests, FG-positive cell number, and real-time PCR assay. Student's t-test was used for lesion volume. All statistical analyses were conducted using the statistical software SPSS (release 12.0). Data are presented as means ±SEM.

#### Results

**Behavioral assessment:** After FLU treatment, improvement of the skilled reaching task in single pellet reaching test was found in the ICH-FLU in comparison with ICH rats. Additionally, the reaching style of ICH-FLU group was more normally than ICH group. Better performance of skilled stepping in ladder test was also shown in the ICH-FLU group than ICH group. However, no significant differences were found in cylinder test and contact placing response among experimental groups.

**Lesion volume and remaining corticospinal tract:** Injection of low-dose collagenase caused small hemorrhage localized the internal capsule (see figure 2). However, lesion volume was not difference between ICH and ICH-FLU group. In parallel, the number of FG-positive neurons in sensorimotor cortex decreased significantly after ICH, but FG-positive neuron number was similar between ICH and ICH-FLU group.

**Real-time PCR:** BDNF and GAP43 are known as growth-promoting factor and involved plastic change of central nervous system. After FLU treatment, mRNA expression of BDNF and GAP43 was significantly enhanced in ICH-FLU animals compared with ICH and Sham animals. These changes were found in the sensorimotor cortex contralateral side to the forced-use side forelimb (ipsilateral side to collagenase injection), but not in ipsilateral side (contralateral to injection).

#### Discussion

In this study, we demonstrated early FLU promote functional recovery of the skilled movement of forelimb (reaching, ladder stepping). Furthermore, upregulation of BDNF and GAP43 was shown in the sensorimotor cortex contralateral to the forced-use forelimb. These data suggested that use-dependent plastic alterations in the forelimb sensorimotor area would be induced by FLU after ICH, causing skilled functional recovery with relation to enhanced expressions of BDNF and GAP-43.

In addition, brain lesion volume and damage of corticospinal tract were mostly unchanged by early FLU. Some previous research has reported immediate FLU after cortical insult enlarged lesion volume and exaggerated functional recovery [4,5]. In this study we used subcortical lesion model, therefore these discrepancy might be related to pathological conditions (cortical vs. subcortical lesion).

**Conclusions**

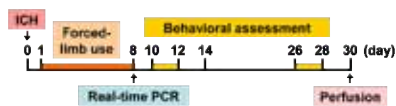
Forced-use of impaired forelimb after capsular hemorrhage induced better recovery of skilled reaching and stepping of the impaired limb. This functional recovery might be related use-dependent alterations of the growth-promoting factors expression in the sensorimotor cortex.

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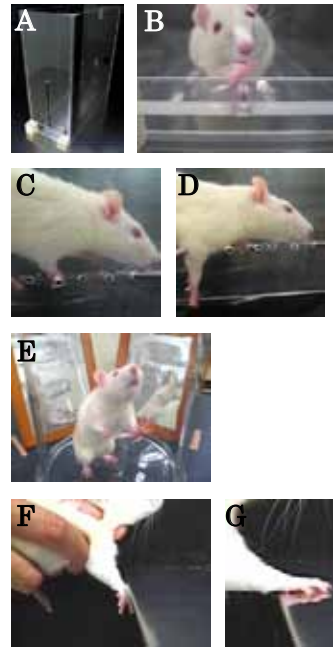
E-Mail: [jshida.akimasa@c.mbox.nagoya-u.ac.jp](mailto:jshida.akimasa@c.mbox.nagoya-u.ac.jp)



**Figure 1. Timeline of the experiments**



**Figure 2. Typical photograph of internal capsule hemorrhage**  
 Hematoxylin and eosin staining 30 days after ICH shows small hemorrhage localized the internal capsule (arrow).



**Figure 3. Behavioral tests**

(A-B) Single pellet reaching test: A shows the apparatus of single pellet reaching test. Reaching to retrieve pellets through a narrow slit by their impaired forelimb was analyzed (B). (C-D) Ladder test: Crossing the 1-m long ladder was videotaped and assessed the rate of correct step and fall. C demonstrated correct step (midportion of limb placed on rung) and D showed fall. (E) Cylinder test: Rats were placed in a plexiglas cylinder 5min, and the percentage of the impaired limb use when touching the cylinder wall was analyzed. (F-G) Contact placing response: Rats were held with the forelimb suspended, and advanced until the dorsum of the forepaw touched the edge of a table (F). Normally, rats show a placing response by extending the forepaw digits and putting them on the table (G).

**RECURRENT RISKS AFTER MILD ISCHEMIC STROKE IN A JAPANESE POPULATION**

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**Introduction**

The high rate of stroke recurrence during several years after first ever stroke has suggested in Hisayama study in Japan. Furthermore, the 70% of ischemic stroke patients have mild motor paresis. However, stroke recurrence has not studied depends on the severity of the paresis, so precise data in the recurrence rate and predictive variables for stroke recurrence in mild stroke remained unknown. Therefore, This study aimed to estimate 3 year cumulative recurrence rates and identified independent predictors of vascular events after TIA and mild ischemic stroke (IS).

**Materials and Methods**

**Subjects:** From December 2005 to September 2006, patients with acute TIA or IS who met criteria of modified Rankin Scale 0-2, no communication disability and emergent admission to a sophisticated acute hospital in Nagoya city district were consecutively enrolled in this study. Patients with age over 80 years old, cardiogenic stroke, severe dementia, psychiatric disorder and extracorporeal dialysis were excluded. During hospitalization, we assessed sex, family history, age, height, weight, stroke subtype, blood pressure, lipid profile, fasting glucose, HbA1c, smoking, alcohol consumption, exercise habits, waist circumference, and ankle-brachial pressure index (ABI). And we also assessed physical activity and salt-intake at 3 month after discharge. We divided into 4 groups followed by physical activity and salt intake. Primary outcome was recurrence of stroke or other vascular events such as myocardial infarction, angina pectoris, and peripheral artery disease during 3 years.

**Statistical analysis:** Continuous variables are expressed as mean standard deviation (SD). Univariate associations between pathophysiological / lifestyle factors and recurrence were assessed using Kaplan-Meier survival analysis, and significance was determined using the log rank test with continuous variables analyzed as median value. Hazard ratios (HRs) for recurrence were determined by univariate Cox proportional hazards regression analyses and variables with a P value of <0.1 at univariate analysis were entered into a multivariate Cox model to determine HRs. All statistical analyses were performed with the SPSS 16.0 software package (SPSS Japan, Tokyo, Japan), and a P value of <0.05 was considered significant.

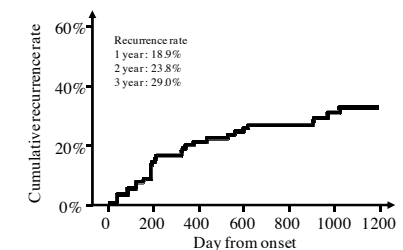
**Results**

**Study population**  
 A total of 102 patients (78 men and 24 women) were successfully followed for 3 years. The mean follow-up period was 1134±80 day. The mean age at study entry was 65.0±10.5 years.

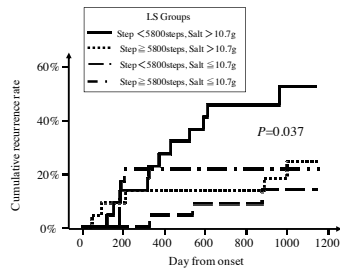
**Outcome at 3 years**  
 Figure 1 shows the Kaplan-Meier estimates of cumulative recurrence rates of stroke for all subjects. Twenty-five out of 102 patients (24.5%) had stroke recurrence, 4 (3.9%) had a coronary event, 2 (2%) died due to cancer, and 1 (1%) had no fixed residence. Eight patients of the 25 who had recurrent strokes had a severe stroke (mRS ≥ 3).

**Pathophysiological factors**  
 In univariate analyses in patients with LVD, abnormal ABI and MetS were significantly associated with higher recurrence rates. In multivariate Cox regression analyses, LVD (HR:2.81, CI:1.13-6.96, P=0.025), abnormal ABI (HR:3.30, CI:1.33-8.20, P=0.009) and MetS (HR:2.67, CI:1.23-5.80, P=0.013) were selected as significant independent predictors for stroke recurrence or cardiovascular events.

**Lifestyle factors**  
 In univariate analyses, higher salt intake was associated with higher recurrence (HR: 2.43, CI:1.04-5.68, P=0.040) and lower physical activity tended to be associated with higher recurrence(HR: 1.10, CI: 0.36 - 3.33, P=0.076). When divided into four groups, the poor lifestyle management group was associated with higher stroke recurrence (HR:1.62, CI:1.10-2.39, P=0.013). In multivariate Cox regression analyses, the poor lifestyle management group remained associated with recurrence. Kaplan-Meier survival curves for lifestyle management are presented in Figure 2.



**Figure 1. Kaplan-Meier estimates of cumulative recurrence rates of stroke. Deaths without stroke recurrence were censored.**



**Figure 2.** Kaplan-Meier estimates of cumulative recurrence rates of stroke according to lifestyle management.

### Discussion

The results of the present study showed that approximately 30% of mild ischemic stroke patients experienced vascular events during the 3 years after onset. To our knowledge, this report is the first study to demonstrate the high recurrence rate in Japanese patients with mild strokes. Moreover, the findings of this study provide several predictive risk factors which include not only pathophysiological factors but lifestyle factors for vascular events.

In the present study, the 3-year risk of recurrence after mild stroke was approximately 30%, exceeding the 10% to 20% rates reported in previous community-based studies which included mild to severe stroke. There might be several reasons for this discrepancy. First, a high proportion of LVD in the present study might have influenced the recurrence rate. For the background behind this higher recurrence rate, increasing prevalence of recurrent risk factors may raise the rate of recurrence. These risk factors have gradually increased during the past decade in Japanese population. Secondly, race may be a possible reason for high stroke recurrence. High stroke recurrence results in increase of severity of the effects of stroke. Indeed, approximately 30% of cases of stroke recurrence in the present study resulted in severe disability.

### Pathophysiological factors for recurrent predictors

The results of the present study indicated that abnormal ABI, MetS and LVD were independent predictive factors for 3-year vascular events. This suggests that advances of atherosclerosis or complexity of traditional risk factors are essential for moderate-term vascular events in mild stroke.

Low ABI, a subclinical marker for lower extremity arterial disease. Previous study showed that ABI is inversely related to traditional risk factors for vascular disease and represents a marker for atherosclerotic changes in other vascular beds. Thus the etiology of abnormal ABI is atherothrombosis. Prevalence of abnormal ABI with other atherothrombosis like stroke in this study has reported to result in poor prognosis. The findings of this study are in line with these previous reports, suggesting abnormal ABI can be

a powerful prognostic factor for stroke recurrence in mild stroke.

MetS shows that obesity with visceral fat accumulation closely associates with atherosclerotic vascular events. Moreover visceral fat obesity is closely related to adipose tissue. Several studies have demonstrated that adipose tissue actively produces a variety of locally and systemically functioning bioactive molecules, including tumour necrosis factor- $\alpha$ , plasminogen-activator inhibitor type-1 and adipocytokine, that also interact in cardiovascular diseases. Especially, adiponectin and leptin effect on increase blood pressure, sympathetic nervous system activity, and expressed within atherosclerotic plaques.[1]

### Lifestyle factors for recurrent predictors

High salt intake and lower physical activity were also indicated as independent predictive factors for stroke recurrence. This result highlights that lifestyle modification can help to prevent vascular events. Results from Japanese population-based prospective cohort studies have showed significant associations between salt intake and stroke incidence[2]. As reported in a previous study, stroke mortality has a strong relationship to dietary salt intake independent of blood pressure. The mechanism responsible for the association between salt intake and strokes is still unknown, but may be related to artery thickness, stiffness and platelet reactivity.

Physical activity also tended to be associated with stroke recurrence. In a meta-analysis, lower physical activity had a 33% higher risk of stroke incidence compared with more active individuals[3]. Although the association between physical activity and strokes has not been well established, it has been clearly demonstrated that insulin resistance and HDL-cholesterol, which contribute to prevent the progression of atherosclerosis, are related to higher physical activity. Improvement of endothelial dysfunction by exercise is also other possible mechanism for the prevention of stroke recurrence.

### Conclusions

The findings of the present study suggest that the recurrence rate in mild strokes has increased in conjunction with increased prevalence of cardiovascular risk factors. The results also suggest that risk reduction through a combination of medication and lifestyle intervention will improve this higher recurrence rate.

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## LOCOMOTOR IMAGERY TRAINING IMPROVES GAIT PERFORMANCE IN PEOPLE WITH CHRONIC HEMIPARETIC STROKE

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### Introduction

Motor imagery practice is an active practice in which the patient imagines or visualizes the performance of a function, a movement, or a task without overt physical therapy.<sup>11</sup> Beneficial effects of motor imagery practice have been supported through studies of brain-imaging. The same areas of the central nervous system are activated during real performances and during motor imagery practice of the equivalent tasks.<sup>12,13</sup> Neuroplasticity of motor activity arising from physical therapy is also reproduced on the neural substrate in the brain involving motor imagery practice of the same activity.<sup>12,14</sup> Recently, motor imagery of gait training for individuals with stroke has been introduced by some researchers.<sup>5,7</sup> Most studies on motor imagery practice are focused on treating loss of upper extremity function<sup>11</sup> and on improving performance in activities of daily living.<sup>15</sup> There are not full studies of intervening in lower extremity and gait dysfunction<sup>5,7,11</sup>

Dickstein et al. (2004) reported remarkable improvement in spatiotemporal parameters after gait-focused motor imagery practice. Locomotor imagery training (LIT) for persons with hemiparetic stroke is a motor imagery training technique that improves gait performance without requiring actual movements by paretic or less paretic limbs. LIT may be beneficial for patients who unable to participate in physical gait training secondary to fatigue, severe paralysis, or improper balance.

The purpose of this study was to evaluate whether motor imagery for gait training improves spatiotemporal parameters on gait, related kinematic gait variables and clinical measures for gait in individuals with hemiparetic stroke.

### Materials and Methods

**Participants:** Thirteen hemiparetic hemiparetic subjects were experimental group. Then we recruited same number of age, lesion, sex, post-stroke duration matched control group subjects. However, two subjects in the control group were drop out before completing post-test. The study was approved by the human research ethics committee of all participating institutions, and all subjects signed an informed consent form after they received information about the study's purpose, procedure, possible benefits and risks, privacy and use of data.

The inclusion criteria were (1) six months or more since stroke onset, (2) ability to walk independently over-ground for at least 10 m with or without use of an ankle foot orthosis or assistive device, (3) absence of any cognitive impairment (scores above 24 on the Mini Mental State Examination),<sup>16</sup> (4) ability to understand verbal instructions, (5) no cerebellar lesion, (6) no significant body or visuospatial hemineglect, and (7) a good ability for imagery functioning (a score of 32 or higher on the revision of movement imagery questionnaire).<sup>17</sup> Table 1 explains the general characteristics of the LIT and control groups

**Interventions:** The subjects in the LIT group participated in four weeks of LIT five-times weekly, with each session lasting 25 to 30 minutes. Subjects in the control group watched documentary television programs for the same period of time.

The first week of the four-week training focused on familiarizing patients with normal gait sequences and

identifying their own problems in gait by studying the differences between the first knowledge of performance of the subject's gait and that of the normal gait. Normal gait sequences were described on the eight phases of gait cycle including initial contact, loading response, midstance, terminal stance, preswing, initial swing, midswing, and terminal swing. The therapist explained the kinematic changes in each phase hip, knee, ankle, and foot. During the last three weeks, the LIT group performed motor imagery practice according to a five-stage protocol: progressive relaxation, external imagery (analysis of task sequences), problem identification, internal imagery, and mental rehearsal. This protocol was developed based on the "active relaxation, imagery, and mental rehearsal (AIM)" strategy, which is commonly used in studies of motor imagery practice for sports.<sup>18</sup> A detail description of the five-stage protocol is shown in Appendix 1.

**Outcome Measures and Analysis:** 3-dimensional motion analysis system and workstation software, Activities-specific balance confidence scale, Berg balance scale, Dynamic gait index.

**Statistics:** Pre-treatment demographic data of the subjects were compared between the LIT and control groups using independent t-tests and chi-square ( $\chi^2$ ) tests. Independent t-tests were used to determine whether the changes from the pretest to the posttest of the spatiotemporal, kinematic, clinical parameters significantly differed between the two groups. An alpha level of  $P < .05$  was used as statistical significance. All statistical analyses were performed using the SPSS statistical package 12.0<sup>1</sup>.

### Results

#### Spatiotemporal parameters

The increase of walking velocity from the pretest to posttest was significantly greater in the LIT group than in the control group ( $P=0.034$ ) (Figure 2). However, the changes between the pretest and posttest for cadence were not significantly different between the two groups ( $P=0.722$ ). The changes of affected and less affected limbs stride length between the pretest and posttest were significantly greater in the locomotor imagery training group (Figure 3). Spatiotemporal parameters except for walking velocity and stride length of both limbs were not significantly different between the groups.

#### Kinematic data

In the affected hip joint, the peak-to-peak extension-to-flexion angular displacement increased from  $31.95 \pm 9.68^\circ$  during the pretest to  $38.08 \pm 5.51^\circ$  after the locomotor imagery training. However, the peak-to-peak joint angular displacement of hip extension-to-flexion in the control group decreased  $1.46 \pm 4.30^\circ$ . In the affected knee joint, the peak-to-peak extension-to-flexion angular displacement increased from  $30.25 \pm 14.27^\circ$  during the pretest to  $41.20 \pm 12.37^\circ$  after the locomotor imagery training. However, the peak-to-peak joint angular displacement of knee extension-to-flexion in the control group decreased by  $3.73 \pm 6.26^\circ$ . This change was significantly greater in the locomotor imagery training group compared with the control group ( $P=0.001$ ). However, the test

<sup>1</sup> SPSS Inc, 233 S Wacker Dr, Chicago, IL, USA 60606

changes of other joint motions were not significantly different between the two groups ( $P>0.05$ ).

In less affected hip joint, the peak-to-peak angular displacement of rotation decreased by  $8.77\pm 10.57^\circ$ , from  $22.18\pm 11.63^\circ$  during the pretest to  $13.41\pm 5.15^\circ$  at the posttest, in the locomotor imagery training group. However, the peak-to-peak joint angular displacement of hip rotation in the control group decreased by  $0.21\pm 9.17^\circ$ . This change was significantly greater in the locomotor imagery training group compared with the control group ( $P=0.048$ ). However, the test changes of other joint motions were not significantly different between the two groups ( $P>0.05$ ).

#### Clinical measures

The changes in the scores of ACTIVITIES-SPECIFIC BALANCE CONFIDENCE scale BBT, and DGI were significantly greater in the locomotor imagery training group compared with the control group (Table 2) ( $P=0.000$ ). The total mEFAP time scores was more decrease in the locomotor imagery training group than in the control group. The change of time scores was significantly greater decreased in locomotor imagery training group compared with control group ( $P=0.035$ ). The performance time in performing all mEFAP (floor, carpet, up & go, obstacles, and stairs) decreased upon termination of the experiment in both the groups. The change of time scores in locomotor imagery training group were significantly greater in all subtests except for up & go compared with those of the control group.

#### Conclusion

The LIP is a cost effective and relatively safe motor rehabilitation intervention for individuals with stroke.<sup>9</sup> Locomotor imagery training maximizes practice opportunities for individuals with stroke with hemiakinesia, low-level functional ability, and little or no movement in the paralyzed limb. Locomotor imagery training is possible form of early gait training despite focusing cognitive stage in motor learning without overt motor output.

#### Key Words

Gait, Locomotor imagery training, Motion analysis, Stroke

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## The effects of an isometric knee extension with hip adduction (KEWHA) exercise on selective VMO muscle strengthening

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### Introduction

In general, patellofemoral rehabilitation involves maximizing quadriceps strength while minimizing the patellofemoral joint reaction forces and stress (Tang et al., 2001). There are many types of quadriceps strengthening exercises. Traditionally, quad sets (Cerny, 1995) and knee-extension exercises have been used (Hanten and Schulthies, 1990). However, these are designed to strengthen the whole quadriceps femoris. The VL muscle is naturally stronger than the VMO muscle; therefore, sustained exercise of both facilitates further strengthening of the VL muscle. In addition, during these exercises, lateral and medial forces are not adequately balanced, and increased lateral displacement of the patella into the trochlear groove occurs (Grabiner et al., 1994). This continued imbalance between the VMO and VL muscles accentuates PFPS (Hanten and Schulthies, 1990).

In this study, we investigated the 4-week training effects of knee extension with hip adduction on onset timing of EMG activity for the VL muscle and VMO muscle in asymptomatic participants. We also compared different methods for the determination of EMG onset times.

### Methods

**Design:** The training effect was evaluated using a one-group pretest–posttest study design. We measured EMG onset times during pre- and post-stair ascending tasks. We determined whether VMO muscle onsets were earlier than those of the VL muscle and whether the times of EMG onset changed after training.

**Participants:** Thirty-three participants with no known lower extremity surgical, musculoskeletal, or neurological history participated in the pretest session of this study. Participants showed faster VL muscle than VMO muscle EMG onset times when they ascended stairs, were selected as participants for the 4-week training using the knee extension with hip adduction (KEWHA) exercise.

**Equipment:** Muscle EMG activities were recorded using a Noraxon Telemetry 2400T (Noraxon Inc., Scottsdale, AZ, USA) with two bipolar Ag–AgCl surface electrodes (Blue sensor, Olstykke, Denmark) during a stair-ascending task. Custom-made stairs were used in this study and consisted of two steps, 20 cm in height, leading to a 60-cm-long platform. The first stair had a run of 28 cm, and the second stair had a run of 90cm. **The stair ascending task:** During the pre-test, the stair-ascending task was used to identify subjects with earlier VL muscle onsets, as described previously (Cowan et al., 2001; Crossley et al., 2004; Hinman et al., 2002). Participants placed the non-dominant foot on the floor, 20 cm away from the dominant foot which was on top of the first step, with the hip and knee joints at approximately 45°; their arms were relaxed by their sides. Both feet were parallel and shoulder-width apart, with no hip rotation and no pelvic rotation. Weight-bearing was mainly on the non-dominant foot, with minimal weight-bearing on the dominant foot where it was in contact with the stair (controlled by an EMG biofeedback program, threshold 1.0–2.0µV). The participants were instructed to ascend with their

non-dominant foot, to keep their trunk in an upright position, and to maintain a steady gaze to the front. Ascending speed was at a rate of 96 steps per min, as paced by a metronome, because it is not known if onset time is affected by different walking speeds. Ascending began randomly when the examiner sounded a bell. Prior to the examination, participants practiced ascending the stairs to ensure they were able to step in time to the metronome. The EMG onsets for the VMO and VL muscles in the stair-ascending task during the pre-test were used to select participants with a 10-ms later onset for the VMO muscle than for the VL muscle (Cowan et al., 2002).

**Intervention:** Subjects were selected on the basis of the pretest participated in the 4-week KEWHA exercise. Participants sat on the Noland–Kickoff table (N-K table) and performed isometric knee extension exercises with 15° hip adduction, as described by Monteiro-Pedro et al. (1999). The subjects performed the exercise 3 days a week with a trainer and 2 days a week at home for a total of 4 weeks. Each session consisted of five trials of 10 repetitions (resting for 5 s between repetitions). One trial involved alternating legs to prevent asymmetry between the strength of the right and left quadriceps muscles. Subjects were seated with their arms folded across their chests and used a back-rest set at a hip angle of 130°. A belt was attached to both the pelvis (from the anterior superior iliac spine [ASIS] line to the chair) and the trunk (under the inferior angle of the scapula to the chair) to prevent motion of the pelvis and the trunk. The chair height was adjusted so that both feet were off the floor.

### Results

#### Effect of the knee extension with hip adduction (KEWHA) exercise on VMO and VL muscles onset time differences

The means  $\pm$  SDs of the EMG onset time differences in the pre- and post-tests are shown in Figure 1. There was no significant difference among the EMG onset time differences for the VMO muscle compared with the VL muscle between the pre- ( $7.72 \pm 95.88$ ) and post-tests ( $-4.22 \pm 40.98$ ) ( $p = 0.63$ ) when data at two SDs of the mean baseline activity were analyzed. Very large variations in individual onset times were observed. When data at three SDs of the mean baseline activity were analyzed, less variation in individual onset times was found, and there was a significant difference in the onset time differences for the VMO muscle compared to the VL muscle between the pre- ( $23.78 \pm 13.61$ ) and post-tests ( $-15.44 \pm 34.70$ ) ( $p = 0.00$ ).

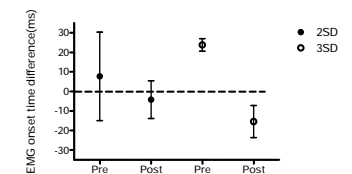


Figure 1. Mean EMG onset time differences (OTD) between VMO and VL during ascending stair task. The dot indicates the mean EMG OTD and the each line indicates the standard deviation (SD). Two and three standard deviations of the mean baseline activity: (2SD, 3SD), VL onset time faster than VMO onset time: (VMO < VL), VMO onset time faster than VL onset time: (VMO > VL).

#### Discussion

The results of this study indicate that participants with a delayed onset time for the VMO muscle showed a decreased difference between VMO and VL muscles onset time after performing the KEWHA exercise for 4 weeks. These results are in accordance with those of Cowan et al. (2002), who conducted studies during a McConnell-based rehabilitation program. However, their study focused on the effects of the position of hip adduction on VMO muscle onset time during the knee-extension exercise, not on a complex exercise program. In addition, our investigations showed that when the threshold for activity was set at two SDs of the mean baseline activity, the results were more variable than those using a threshold of three SDs.

#### Knee extension with hip-adduction (KEWHA) exercise

Hip adduction exercises can be used to selectively strengthen the VMO muscle (Coqueiro et al., 2005). Previous studies combined hip adduction and open- or closed-chain exercises performed by asymptomatic subjects. The VMO muscle exhibited a significantly greater amount of activity than the VL muscle during open chain hip-adduction exercises (Hanten and Schulthies, 1990). No change in the activity of the VMO muscle was found during a combination of isometric hip adduction and open-chain knee extension, although VL muscle activity was significantly less than that of the VMO muscle. These data indicate that extensor mechanisms can reinforce decreased VL muscle activity (Rice et al., 1995). Earl et al. (2001) produced more overall activity in the quadriceps by combining isometric hip adduction with a closed-chain mini-squat. However, this exercise did not specifically support the activity of the VMO muscle, as VMO and VL muscles activity increased by the same amount. Reports on the activation of the VMO and VL muscles during open- or closed-chain exercises cannot be applied to our study, as they are not directly related to it. We did not consider open- or closed-chain exercises, as we modified the methods of traditional quadriceps strengthening exercises that use extension of the knee joint from 90° flexion to full extension on the N-K table. Furthermore, the purpose of our study was to investigate the differences in onset times that arise from changes in motor control as well as the differences in quadriceps activation after exercise. Herrington and Pearson (2006) found no significant differences in onset times between open- and closed-chain knee extension exercises. This suggests that the interactions between the VMO and VL muscles remain fairly constant irrespective of the type of exercise.

#### Conclusion

The knee extension exercise is an established 'self-exercise' used for knee pathology treatment and strengthening exercises. However, if therapists do not adequately instruct the subject on proper positioning, the patella will receive a negative impact due to an imbalance in the activation of the VMO muscle and VL muscles. This study describes the 4-week training effects of the KEWHA exercise, which was used in an attempt to decrease the onset-time difference between the VMO muscle and VL muscles. This is one factor that influences the lateral tracking of the patella. As a result of the 4-week training period, the onset time difference between the VMO and VL muscles was decreased. In our study, the KEWHA exercise removed

one of the PFPS risk factors that reduced the onset time difference between the VMO muscle and VL muscle. Furthermore, we suggest that the use of a correct KEWHA exercise may reduce the risk of lateral tracking of the patella.


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*Occupational Therapy (13:30 ~16:00 Lecture Room 4)*

*Session chairs: Prof. Yuji Sawada  
Prof. Eun Young Yoo  
Prof. Kunifumi Suzuki*

Overview of Occupational therapy in Japan

ITO Emi (Occupational Therapist, PhD)

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This short lecture will focus on Occupational Therapy (OT) in Japan. Contents of the lecture are as follows. Firstly, a brief history of OT will be introduced: 1) precursors of OT, 2) the establishment of 'the law of PT and OT', 3) paradigms. Secondly, the characteristics of Occupational Therapists (OTs) and their practical areas are shown: 1) physical dysfunctions, 2) mental disorders, 3) pediatric and developmental disorders, 4) geriatric disorders. Thirdly, educational systems for OTs are informed: 1) schools, 2) educational criteria, 3) post graduate and lifetime education. Finally, the future of OT will be discussed.

The first school for OTs was established as a three year diploma course in 1963. The first national examination for the license of OT was held and Japanese Association of Occupational Therapists (JAOT) was formed in 1966. JAOT joined WFOT an associate member in 1970 and then, became a full member in 1974. According to the roster of OTs registered in 2010 (JAOT, 2010), there are 53,080 OTs in Japan. There are more female OTs than male – the ratio is two to one - and almost a half of OTs are under 30 years old (JAOT, 2010).

Seventy percent of OTs are working in clinical settings (hospitals and clinics) and the rest of them are working at geriatric facilities or welfare institutions in their communities (JAOT, 2010). Major practical fields are physical (36%), geriatric (25%), mental (15%), and developmental (7%) areas (AJOT, 2005). OT is covered by both national health insurance and national long-term care insurance.

There are now 171 schools (187 courses) with around 7,000 OTs graduating each year. Various kinds of schools are being founded, such as 3 year diploma course, 4 year diploma course, 3 year associate degree course, and 4 year degree course. In addition, 32 Master courses and 22 doctorate courses have been firmly established (JAOT, 2009).

In recent years, we are striving for increasing OT in the community including private homes, group living, institutions as permanent living places, public schools, health centers, and local government beyond hospitals. Super aged society (aged proportion: 22.7% in 2009), and the philosophy of independent living and normalization, encourage us to move into the community where we will be able to contribute further to expanding aspects of OT: not only aiding the recovery of functional components, but also helping clients engage in meaningful occupations, assisting with home modifications, supporting accommodation to own environment, adapting devices and aids, and collaborating with other professions.

The ultimate purpose of OT is for all the people who require OT to live their lives in hope and with value.

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## The Beginning and Development in the Field of Occupational Therapy in Korea

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The field of occupational therapy in Korea began in 1950 for the purpose of treating injured persons during the Korean War. Western missionaries took a primary role in providing the foundation of occupational therapy in Korea. The national licensure examination system was implemented in 1969 and has been given yearly to the present time with an average passing rate of 78%.

Undergraduate education of occupational therapists began in 1979 at Yonsei University and currently, there exist 27 3-year colleges and 21 4-year colleges and universities that provide education in occupational therapy. Currently, approximately 1,000 college graduates are being admitted as first-year college students each year, with a total of 5,403 registered occupational therapists. The average employment rate for occupational therapists between 2007 and 2009 stands at 85%.

In regards to the gender distribution of occupational therapists, 82% are female and 18% are male. With the rapid installment of occupational therapy departments across the nation, the average age group of occupational therapists has also declined such that 69% of occupational therapists are currently in their 20's. Occupational therapists are working in diverse locations such as rehabilitation hospital, medical centers, local clinic, welfare facilities, and institutes. A majority of occupational therapists (67%) are employed in rehabilitation hospitals and medical centers, working primarily with individuals with physical disabilities and only a minority working in the mental-health field.

The Korean Association of Occupational Therapist (KAOT) was established in 1993 and has been responsible for securing members' professional rights, entry into diverse professional fields, and continuing education of its members. KAOT and a number of occupational therapy undergraduate programs have received accreditation from the World Federation of Occupational Therapy (WFOT) in 1998. Currently, there are 5 colleges whose curriculum has been accredited by the WFOT. The Korean Society of Occupational Therapy began its journal publication in 1993 for the purpose of qualitatively improving research in the field of occupational therapy in Korea and is being published quarterly. The Journal of Korean Society of Occupational Therapy has been accepted into the Korea Research Foundation citation index, the most prestigious journal evaluation institute in Korea.

The KAOT is currently putting in all of its effort into passing appropriate federal regulations that will open doors for occupational therapists to enter the field of mental health, in addition to collaborating with other disciplines and working to provide and implement policy guidelines and systems to meet the growing elderly population similar to Japan.

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## BIOLOGICAL OBSERVATION DURING THE DAYTIME OF ELDERLY PATIENTS WITH ADVANCED DEMENTIA CARED FOR WITH AND WITHOUT ARTIFICIAL NUTRITION BY PERCUTANEOUS ENDOSCOPIC GASTROSTOMY

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### Introduction

One of the interventions for patients with a poor oral intake has been enteral tube feeding by percutaneous endoscopic gastrostomy (PEG). [1] Although the potential benefit of PEG to improve a patient's nutritional condition has been emphasized, the role of treatment in patients with advanced dementia has recently been debated, [2] and the number of systematic studies on demented patients treated with PEG is still limited.

The objectives of the present study were to overview the activity of elderly patients with advanced dementia cared for with and without artificial nutrition by PEG in a care facility. Focusing on awake and sleep states and oral movement of the patients, we continuously recorded and quantified the awake/sleep states and spontaneous swallowing movement during daytime in demented patients.

### Materials and Methods

Twenty-six subjects participated in the present study. Thirteen demented patients (one male and twelve females, mean age: 84.0  $\pm$  6.2 (years,  $\pm$ SD) years) received artificial nutrition by PEG (PEG-patients), and 13 patients (one male and twelve females, mean age: 80.2  $\pm$  4.7 with dementia took meals orally (OR-patients). PEG- and OR- patients all suffered from advanced dementia.

PEG-patients could not answer any question in the minimal state examination (MMSE), while the mean MMSE score was 7.8 in OR-patients with statistically difference from that in PEG-patients (t-test,  $p < 0.01$ ).

We selected Wednesday as the day of the experiment, since bathing and outside activities were not scheduled for that day. We carefully observed and described each subject's behaviors every 10 min, such as eating, talking, and attending activities.

EEG signals were recorded from two scalp areas, Oz and C3, with referential electrodes of linked earlobes, using surface electrodes, which were 7-mm silver-silver chloride disc electrodes. Those recording areas were selected to observe alpha basic activity and sleep-specific responses, the vertex potential and sleep spindle. EMG signals for oropharyngeal movements were recorded using a pair of surface electrodes. Recording and reference electrodes were placed on the thyroid cartilage and chin along the midline, respectively, and an earthing electrode was positioned on the midpoint of the left clavicle. EOGs were recorded from the right eye, placing electrodes 2 cm below the infra-orbicular edge and 2 cm lateral to the lateral canthus of the right eye.

The Biological signals were evaluated every 5 min. Awake and sleep states, i.e., being awake or asleep, were investigated based on EEG signals. Awake/sleep states were evaluated, but we partially modified the score with EOG data. The period of time in awake/sleep states was expressed as a percentage of the recording period, since the total recording period varied in each subject, as described in the results. From EOG signals, the period of time spent blinking or showing step-wise rapid (saccadic) eye movement, corresponding with the full-awake state, was measured.

We showed comparisons of values recorded between OR- and PEG- patients. The time period of awake/sleep states was compared between the groups using one-way analysis of variance (ANOVA) with a post-hoc test (Fisher's protected least significance test, Fisher's PLSD test). The swallowing frequency was compared between PEG- and OR-patients using a t-test. Since the dementia score, MMSE, was zero in all PEG-patients, the effect of MMSE score on the awake state and swallowing frequency was tested. Correlations between the MMSE score or BI and amount of time in an awake state, and between the MMSE score or BI and number of spontaneous swallowing movements were tested in OR-patients, using Bartlett's test. P-values less than 0.05 were considered to be significant.

The awake/sleep score was determined every 5 min as: FA: 3, RA: 2, Sleep (S1 or S2): 1. The mean awake/sleep score every 20 min from 7:40 to 17:00 was calculated in each subject, and the value every 20 min was compared between PEG- and OR-patients, using the t-test.

### Results

EEG, EMG, and EOG signals were successfully obtained from all patients. Some periods were omitted from analysis due to physical intervention, such as transferring, toilet, etc., and the total recording period was 412 (6 hours and 52 min)  $\pm$  42 min (SD). Since a light-sleep state (S2) was defined by spontaneous sleep spindles, it was difficult to identify the onset and end of S2 during the recording period. Thus, FA, RA, and sleep (S1+S2) states underwent to statistical analysis.

There was a significant main effect ( $F(5, 72), p < 0.001$ ) of the sleep state, and there was a significant difference in the amount of time in FA ( $p = 0.0041$ ) and S1 ( $p = 0.0013$ ) states between PEG- and OR- patients (Fig. 1).

The spontaneous swallowing frequency was also significantly lower in PEG-patients ( $0.1 \pm 0.3$ , times per hour) than in OR-patients ( $3.4 \pm 5.2, p < 0.001$ ). There was no significant correlation between the MMSE score or BI and period of time in an awake state (MMSE:  $r = 0.227, p = 0.465$ , BI:  $0.406, p = 0.173$ ), and between the MMSE score and number of spontaneous swallowing movements ( $r = 0.435, p = 0.140$ ) in OR-patients. However, the BI and number of spontaneous swallowing movements correlated significantly in OR-patients ( $r = 0.863, p < 0.001$ ).

Sequential changes in the awake/sleep score during the day for all subjects are shown in Fig. 2. The score was significantly greater, i.e., more awake, in OR-patients from 7:40-8:40, 9:40-10:00, 11:20-11:40, 12:00-13:00, and 16:40-17:00 (Fig. 2).

### Discussion

The present study investigated actual awake/sleep states and the swallowing frequency in demented patients with and without artificial nutrition in a care facility during the day. To our knowledge, such detailed observations have not hitherto been reported. Besides decreased physical and mental activities in patients with advanced dementia, present study showed: 1) shorter awake and longer sleep states, 2) the frequency of spontaneous swallowing was markedly lower in PEG-patients,

while the value correlated with BI in OR-patients; 3) The length of time in an awake state and swallowing frequency were not always related with the MMSE score in OR-patients; 4) the awake/sleep pattern during the daytime was variable in PEG-patients, and meal times were the limited periods keeping OR-patients awake consistently.

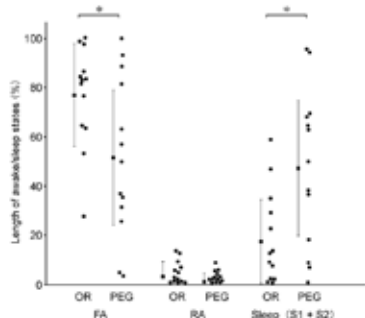


Figure 1: Length of awake/sleep states during the recording period in demented patients undergoing artificial nutrition by PEG (PEG) and patients with dementia who took meals orally (OR). The value was significantly different in the full-awake state (FA) and sleep states (S1 and S2) between OR- and PEG-patients (\* p < 0.01). There was no difference in the value for the rest-awake (RA) state. A solid square (■) and vertical bar indicate the mean and standard deviation, respectively in each patient group.

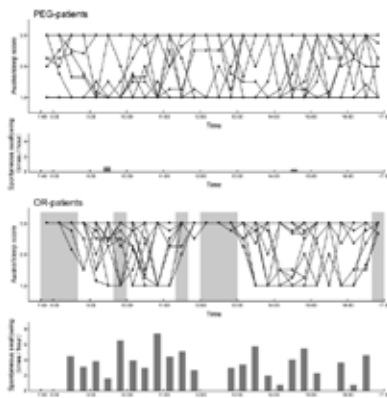


Figure 2: Sequential changes in the awake/sleep score (Full-awake: 3, Rest-awake: 2, and Sleep: 1), and spontaneous swallowing movement (times per hour, solid bars) for PEG- and OR- patients during the daytime. Gray areas indicate the time period in which the sleep score was significantly higher in OR- than in PEG-patients (p<0.05, t-test). Values for each patient are linked by lines. Each value for swallowing was average value for all subjects. Swallowing movements during meals were omitted.

An awake state was maintained during breakfast and lunch times in OR-patients, significantly differing from the awake/sleep states in PEG-patients. However, in other words, awake/sleep states were not different between PEG- and OR-

patients during period between meals. For those patients with advanced dementia, meal times could be the only time keeping them awake, but there was no such opportunity for PEG-patients. Apart from nutritional cycles, the awake/sleep rhythmicity was important regarding the cognitive function in patients with and without dementia. [3] Due to the lack of daytime rhythmicity set by meals, another intervention to maintain the awake/sleep rhythmicity might be considered for PEG-patients.

Concerning the spontaneous swallowing frequency, He et al. [4] reported that a high-risk group for aspiration pneumonia showed a depressed swallowing function. Since spontaneous swallowing movements convey oral bacteria into the stomach and regulate the quality and quantity of oropharyngeal secretions in the pharynx, such movements are an important mechanism for pneumonia prevention. [5] Most of the OR-patients and all PEG-patients in the present study were within the high-risk range for aspiration pneumonia, and some of them had a history of it. Although oral intake was prevented, aspiration pneumonia remained as one of the major problems in PEG-patients. [6] It is of no doubt that pneumonia has been a leading cause of mortality among patients in care facilities, especially demented patients with a poor oto-pharyngeal function, [7] and PEG intervention seems to prevent a direct risk of poor nutrition. However, frequency of spontaneous swallowing more decreased as lower score of BI, and it almost diminished in PEG patients with zero of BI. From the aspect of the spontaneous swallowing frequency, we considered that the risk of pneumonia could never be reduced in PEG-patients.

### Conclusions

We reported awake/sleep states and spontaneous swallowing movements during the daytime in demented patients cared for with and without PEG intervention. In the present study, serial recording of biological activity could give us precise knowledge of what happens in patients with advanced dementia and those after PEG intervention. It also gave us and information regarding actual sleep pattern and temporal characteristic of a risk factor of aspiration pneumonia, spontaneous swallowing, and their relationship with MMSE and BI in those patients.

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## OCCUPATIONAL THERAPY AS A CAREER: KOREAN UNDERGRADUATE OT STUDENT'S PREFERENCES AND CONCERNS REGARDING THEIR FUTURE AS OCCUPATIONAL THERAPISTS

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### Introduction

With the growing number of newly graduating occupational therapy students, there has been recent concern regarding the future job market for occupational therapists and emphasis on the need to reorganize the areas of OT service in Korea. The present study analyzed Korean undergraduate occupational therapy (OT) students' preferences and concerns regarding their future as occupational therapists using a survey method. The primary purpose of this study was to examine (1) OT students' reasons for choosing occupational therapy as their major, (2) their thoughts and beliefs concerning their future as occupational therapists, and finally (3) to contribute to the development of the field of OT in Korea by providing information regarding students' preferred fields of employment and their professional interest areas.

### Materials and Methods

The participants in the study were 1,666 Korean undergraduate OT students from 19 universities and colleges across Korea. A packet of questionnaires was given to students. The questionnaire was made up of three different areas: (1) students' demographic characteristics in addition to reasons for selecting OT as their major, (2) concerns and anxiety regarding their future, and finally (3) their current preferred field of employment and professional interest areas. Demographic variables included variables such as year in college, student group type, gender, age, and religion. Students were asked to choose among 7 reasons that led them to choose OT as their major. 16 items on a 4-point Likert scale were created to address students' concerns regarding their future as occupational therapists. Lower score indicated greater future career concerns. Students also responded to a question regarding their preferred field of employment. Data was analyzed using SPSS 12.0.

### Results

#### (1) Demographic Characteristics

A total of 723 universities and 606 colleges that had departments of occupational therapy were represented in the present study. The sample consisted of 352 (26.5%) male and 977 (73.5%) female students. The sample consisted of 483(36.3%) freshmen, 457 (34.4%) sophomore, 335 (25.2%) juniors, and 54 (4.1%) seniors. The student group type consisted of 1139 (85.7%) currently enrolled students, 133 (10%) students returning to school after taking time off, 28 (21%) transfer students, and 27 (2%) readmission students.

#### (2) Reasons for Selecting OT as their Major

The results indicated that 675 (50.8%) OT students chose OT as their major following recommendations made by others (parent, teacher, sibling). 654 (49.2%) OT students replied that they chose the major on their own. The principle reason for recommendation and selection was to work in a professional field or to secure employment. 476 (70.4%).

#### (3) Thoughts Concerning their Future

The results indicated that 693 (52.1%) OT students have high career confidence, 504 (37.9%) OT students have average career confidence and 117 (8.8%) OT students have low career confidence when they first entered the department. However, 362 (27.3%) OT students reported high career confidence, 603 (45.4%) OT students average career confidence and 352 (26.4%) OT students low career confidence presently. Such results indicate that career confidence is lower in the present than in entering school. Female students reported greater future career concerns than male students. Freshmen reported greater future career concerns than sophomores, juniors, seniors. 831 (62.5%) OT students hoped to transfer to another academic department.

#### (4) Preferred Field of Employment and Professional Areas of Interest

The results indicated that most 1157 (36.4%) OT students were most interested in the areas of mental health, physical disability, and autism-spectrum disorders, respectively. The most desired place of work following graduation was in a medical center / teaching hospital (n=908; 68.3%). This preference held true in students' response to the question, "Where would you like to be working in 10 years?". OT students indicated that would prefer working with the children (n=792; 40.6%), followed by the adult population (n=632; 32.3%) and elderly (n=425; 21.7%).

#### (5) Additional Analysis: Relationship between OT Students' Demographic Characteristics and Preferences and Concerns Regarding their Future

First of all, there was a meaningful effect of gender on future career concerns. Female students reported greater future career concerns than male students (t = 3.46, p < .05). Second, there was a meaningful positive correlation between year in college and career concerns. More specifically, the higher the year in college, students reported greater future career concerns (r = -.126, p < .01).

### Conclusions

In conclusion, the results of the present study indicate that Korean OT undergraduate students' degree of future career concerns is affected by factors such as gender and year in college. This suggests the need for departments in OT to tailor their education and student mentoring depending on students' gender and year in college. Also, this study indicates that the preferred interest or work field of Korean OT undergraduate students was mostly limited to medical institutions. Therefore, further guidance is required to expose OT students to more diverse areas of interest and work field.

## ALTERATION OF TIME PERCEPTION IN YOUNG AND ELDERLY PEOPLE DURING JIGSAW PUZZLE TASKS WITH DIFFERENT COMPLEXITIES

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### Introduction

Recent studies have suggested that subjective time perception is strongly linked to the quality of activity performed. [1] Thus, time perception is an important factor during occupational therapy, which might promote clients' positive experiences. [2]

Abnormal time perception has been reported in patients with dementia, [3] but little is known regarding the neuropsychology and pathophysiology of time perception. Contrarily, the time perception of clients may give us information regarding their physical and mental states. We suggest that time perception is informative to evaluate clients' psychological and pathological conditions. Previous studies reported time perception among ages and tasks, [4] but the results were not consistent.

In the present study, focusing on healthy people, we investigated time perception in young and elderly people during tasks. We discussed the psychological mechanisms of time perception during tasks based on the hypothesis of Larson and von Eye. [2] The objective of this study was to clarify the characteristics of time perception in the clinical situation of occupational therapy (OT), along with the performance of a globally well-known jigsaw puzzle tasks.

### Materials and Methods

Twenty-eight elderly (8 men and 20 women, mean age:  $69.9 \pm 6.2$  (SD) years, range: 60-85) and thirty-one young (8 men and 23 women, mean age:  $21.9 \pm 1.4$ , range: 18-24) participants were involved in the present study. All participants had no motor, sensory, or cognitive symptoms, and no history of neurological diseases. The elderly subjects had no behavioral or clinical symptoms of dementia or mild cognitive impairment, and their scores in the mini-mental state examination were in the normal range over 28 points. They had no detectable visual, auditory, or speech disturbance on performing jigsaw puzzle tasks, and they had no speech disturbance on answering questions asked by examiners.

Participants were asked to complete two jigsaw puzzles with different complexities, and a time estimation test was applied during the puzzle tasks. All tasks were performed in a quiet room in the afternoon.

Time estimation tests were performed during the two puzzle tasks. One was a simple task with 24 pieces, and the other was a complex task with 54 pieces. Participants were randomly divided into two groups; those who performed the 24- or 54-piece puzzle first (cross-over design). They were specifically instructed to concentrate to complete the puzzle tasks. Examiners told participants to start a puzzle task on hearing a beep sound and to stop the task 17 minutes and 17 seconds after the onset on hearing another beep sound. The fixed duration of the tasks, 17 min and 17 sec, was chosen as an unpredictable time.

Just after stopping the puzzle task, participants were asked to verbally answer how long in minutes and seconds they thought they had spent on the task. After the two puzzle and time estimation tasks, subjects were asked to answer a

questionnaire regarding interests and feelings during the puzzle tasks.

We measured the time required to complete each puzzle task and, then the ratio of the subjective to absolute duration of time, the duration judgment ratio (DJR), [6] was calculated for each task. A DJR of 1.0 indicates that the participants estimated time was equal to the actual time, whereas a DJR of less than and greater than 1.0 indicates under- and overestimation of time, respectively. The values of tasks obtaining groups performing the 24- or 54-piece task first were averaged. The time required to complete the puzzles and DJR values were compared using two-way (groups and tasks) analysis of variance with Tukey-Kramer's tests for multiple comparisons. For subjective feelings, the DJR values were compared among the four grades of interest and among the four different feelings employing Mann-Whitney tests. A p-value less than 0.05 was considered significant.

### Results

Twenty-six and two elderly subjects completed 24- and 54-piece jigsaw puzzles, respectively, and all young subjects completed both puzzles. Excluding subjects who did not complete the puzzle, the elderly group took  $486.14 \pm 209.87$  (mean  $\pm$ SD) and  $1,035.32 \pm 6.24$  sec, and the young group needed  $127.29 \pm 33.77$  and  $466.65 \pm 115.28$  sec for the 24- and 54-piece jigsaw puzzles, respectively (Fig. 1). The elderly group required significantly longer time than the younger group ( $F [1, 114] = 444.5, p < 0.001$ , ANOVA). The DJR values are shown in Fig. 1. Both elderly and young subjects estimated that tasks took a longer time than they actually did, i.e., the DJR value was more than 1.0. The effect of group on the DJR was not significant in each task ( $F [1, 114] = 0.75, p = 0.385$ ). However, the effect of the task on the DJR was significant ( $F [1, 114] = 31.75, p < 0.001$ ), and the value was greater in the 24- than in the 54-piece task in both groups ( $p < 0.01$ , Tukey-Kramer's test).

The number of subject, who felt the task was difficult was higher in the elderly group than in the young group in each task ( $p < 0.05$ , Mann-Whitney tests). The number of young subject who answered that the task was interesting was greater than in the elderly subjects for the 54-piece task ( $p < 0.05$ ).

Among the sub-groups of each grade of interest, the DJR was smaller in the group with "much interest" than in that with "little interest" for the 24-piece task ( $p < 0.05$ , Mann-Whitney tests), but there was no difference for the 54-piece task. Subjective feelings of "engaging", "difficult", and "enjoyable" had no effect on the DJR.

### Discussion

The present results can be summarized as: 1) subjects estimated that tasks took a longer time than they actually did, 2) subjects estimated a longer time for the 24- than 54-piece task, 3) there was no difference in the time estimation between young and elderly groups, although the feeling of difficulty was significantly different between the groups, and 4) subjects interested in the task estimated a time shorter than those not interested in both the young and elderly groups. The present

study clarified behaviors in young and elderly subjects regarding time estimation, which have been theoretically reported. [2]

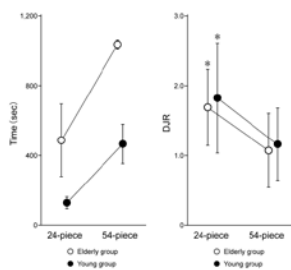


Fig. 1: The mean time required for the puzzle tasks (left). The elderly group required a significantly longer time than the younger group for both tasks ( $p < 0.01$ , ANOVA). The mean duration judgment ratio (DJR) during the tasks (right). Both elderly and young subjects estimated a time longer than the actual time during the tasks. The effect of group on the DJR was not significant in each task, while the effect of the task on the DJR was significant ( $p < 0.001$ ). The value was greater in the 24- than 54-piece task in both groups (\*  $p < 0.01$ , Tukey-Kramer's test). Each vertical line indicates a standard deviation.

The DJR value was more than 1.0 in both tasks in both young and elderly groups. This indicated that the subjects felt that the time passed slower than it actually did during the tasks. Between the tasks, the 24-piece task had a greater effect on the prolonged time estimation of subjects than the 54-piece task in both groups. These results regarding the subjective estimation of time are generally in line with those in previous studies. [5]

Coelho et al. [4] reported a change in the estimation and production of time intervals with aging, suggesting an association of aging with a faster "internal clock", which resulted in a longer subjectively estimated time than the absolute time, at least over a short duration around 10 sec. Since our time estimation task required a relatively long time, 17 min and 17 sec, it might not be possible to compare the results to previous ones.

One explanation concerning the effect of aging in the present study may be plausible, based on Larson's model of time perception (Fig. 2). [2] Time perception could be explained by the task content, as well as psychological condition. When a task during time estimation was successful, time passed slowly, i.e., a low DJR. On the other hand, when the task was challenging, subjective time passed quickly. When the task was difficult being beyond subjects' ability, the estimated time again became long. During the 24-piece task, both young and elderly subjects successfully performed the task. Young subjects were challenged by the 54-piece task, and their subjective time estimation was shorter than for the 24-piece task. However, for elderly subjects, the 54-piece task was difficult to perform, and the time estimation was long in the range of "beyond ability" (Fig. 2). We considered that it was important to take into account not only the absolute complexity of tasks, but also subjects' feelings during tasks or how they performed in them.

Similarly, another novel result in the present study was that the subjects' feelings of interest in the task affected the time estimation. This result suggested that time perception during a task, or an occupation, could be modified by one's

mental attitude and manner, and that involvement with interest in a task shortens a subject's time perception. Besides relative factors should be important to manage the time perception of clients during occupational therapy, since spending a fruitful time and the quality of daily activities could be linked to time perception. [2]

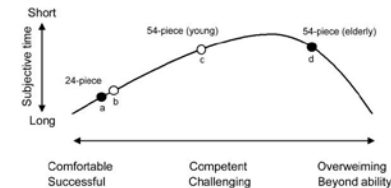


Fig. 3: Hypothetical explanation of the results for time estimation in young (open circles) and elderly (solid circles) subjects of the present study (the time estimation curve is a modified figure of Larson and von Eye. [2] For the 24-piece task, both groups performed it successfully (a and b). For the 54-piece task, young subjects were challenged (c), while it was too difficult for elderly subjects to complete (d).

### Conclusions

In conclusion, we investigated the characteristics of time perception in the clinical situation of occupational therapy along with the performance of jigsaw puzzle tasks. Subjective feelings modified time perception in both young and elderly subjects, and a part of the results could be explained by a model of time perception in an occupation. Occupational therapists can consider these features. As Larson and von Eye [2] pointed out, if the client perceives a daily task as disinteresting, there are shifts in mindfulness during the task or an increase in cognitive and emotional involvement. Conversely, if a task of daily living is too challenging, the therapist might redesign the task by reducing the complexity and increasing the client's skill. By knowing characteristics of time perception in each client, occupational therapists could use such knowledge to use the time for interventions effectively, as well as employ it as personal information on the client.

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## EFFECTS OF SCHEDULED EXERCISE PROGRAM COMBINED WITH FORCED USE ON UPPER EXTREMITY FUNCTIONING IN INDIVIDUALS WITH HEMIPLEGIA

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### Introduction

Although individuals with hemiplegia need continuous therapy to improve upper extremity functioning, they spend most of their time alone and not exercising spontaneously outside the outpatient clinic setting (Bernhardt, Chan, Nicola, & Collier, 2007). The goal of the present study was hence to suggest the potential effectiveness of an exercise program that can encourage client's voluntary participation while minimizing therapist involvement. More specifically, the purpose of this study was to investigate the effects of scheduled exercise program combined with forced use in improving upper extremity functioning in individuals with hemiplegia.

### Materials and Methods

**Participants:** Two males and one female diagnosed with chronic stroke participated in the present study. The inclusion criteria were as follows: (1) a minimum of 6 months post single unilateral stroke and currently neurologically stable, (2) no significant cognitive deficits as indicated by a score > 25 on the Korean Mini Mental Status Examination (MMSE-K) (Kwon & Park, 1989), (3) ability to actively extend at least 10° at the affected metacarpophalangeal and interphalangeal joints and 20° at the more affected wrist and (4) no balance problems that may compromise safety.

**Design:** A-B-A'-C reversal design was used in the study.

**Setting:** Home-based.

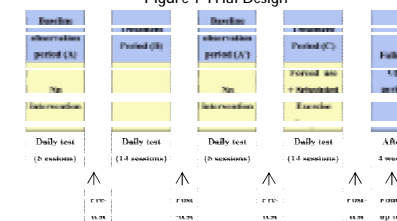
**Intervention:** The treatment process consisted of two conditions: forced use only (B intervention period) and individual customized scheduled exercise program in addition to forced use (C intervention period). During the B and C intervention periods, participants had their healthy arm immobilized by wearing mittens for 6 hours such that they were not able to manipulate objects using the non-affected hand. During the C intervention period, exercise programs were conducted 14 times along with forced use according to the fixed schedule. A total 10 activities (5 tasks targeting arm functioning and 5 tasks of activities of daily living: ADL) were adopted which differed across the three participants based on their level of functioning in the affected upper extremity. The present researcher selected tasks for improving arm functioning based on individual's performance with the goal of improving functioning of the shoulder, elbow, hand, and finger. ADL tasks were determined based on information gathered from participant and caregiver interviews, with the goal of selecting tasks that were deemed essential in minimizing other's help.

**Outcome measures:** The Box and Block Test (BBT) (Cromwell, 1976) and the Wolf Motor Function Test (WMFT) (Wolf, Lecraw, Barton, & Jann, 1989) were used to assess motor function of the upper extremity. Participant's rate of performance of assigned activities was determined by the number of assigned activities accomplished on a day divided by the total of 30 activities multiplied by 100. To determine the rate of implementation of scheduled exercise activities, the number of scheduled exercise tasks implemented on a day divided by the total of 10 assigned tasks multiplied by 100. The Motor Activity Log (MAL) (Page, Sisto, & Levine, 2002) was

used to assess ADL. In order to examine the psychosocial status of the participants, the Self-Esteem Scale (Rosenberg, 1965) and the Psychosocial Well-Being Index Short Form (SF-PWI) (Chang, Cha, Won, & Koh, 2001) were measured pre- and post-intervention.

**Data analysis:** The scores for each participant on the above-mentioned outcome measures were visually analyzed using graphs.

Figure 1 Trial Design



### Results

The results of the present study can be summarized as follows:

1. Performance rates to measure motor functioning of Participant 1, 2, and 3 were 99.0%, 22.1%, and 74.6%, respectively, on the BBT. Compared to the baseline period, the number of wood blocks which were moved during the period of intervention improved. The degree of performance on the WMFT showed improvement in the post-intervention period C compared to the baseline A period and the amount of time needed to complete the tasks also decreased.
2. Performance rates on tasks of ADL for Participant 1, 2, and 3 were 57%, 113%, and 48.8%, respectively, markedly improving from the baseline period. Results from the MAL indicate improvement in the use and quality of movement in the post-baseline C period compared to the baseline A period.
3. During the intervention C period, the implementation rates of ADL schedule for Participant 1, 2, and 3 were 85%, 70%, and 90%, respectively.
4. On measures of psychosocial status, improvement in self-esteem was found for Participant 1 and 3, while their sense of psychosocial stress declined. For Participant 2, the self-esteem score declined and the psychosocial stress remained unchanged.

#### Conclusion

The results of the present study indicate that scheduled exercise program combined with forced use had positive effects on improving upper extremity functioning and activities of daily living in persons with post-stroke hemiparesis in the home setting. Such scheduled intervention program also showed partial effects on improving psychosocial status. Furthermore, the present study also suggests that chronic stroke patients can take part in exercise programs on their own within the home setting and improve upper extremity functioning without therapist intervention.

#### Key Words

Activities of Daily Living, Arms Function, Forced Use, Schedule, Stroke

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