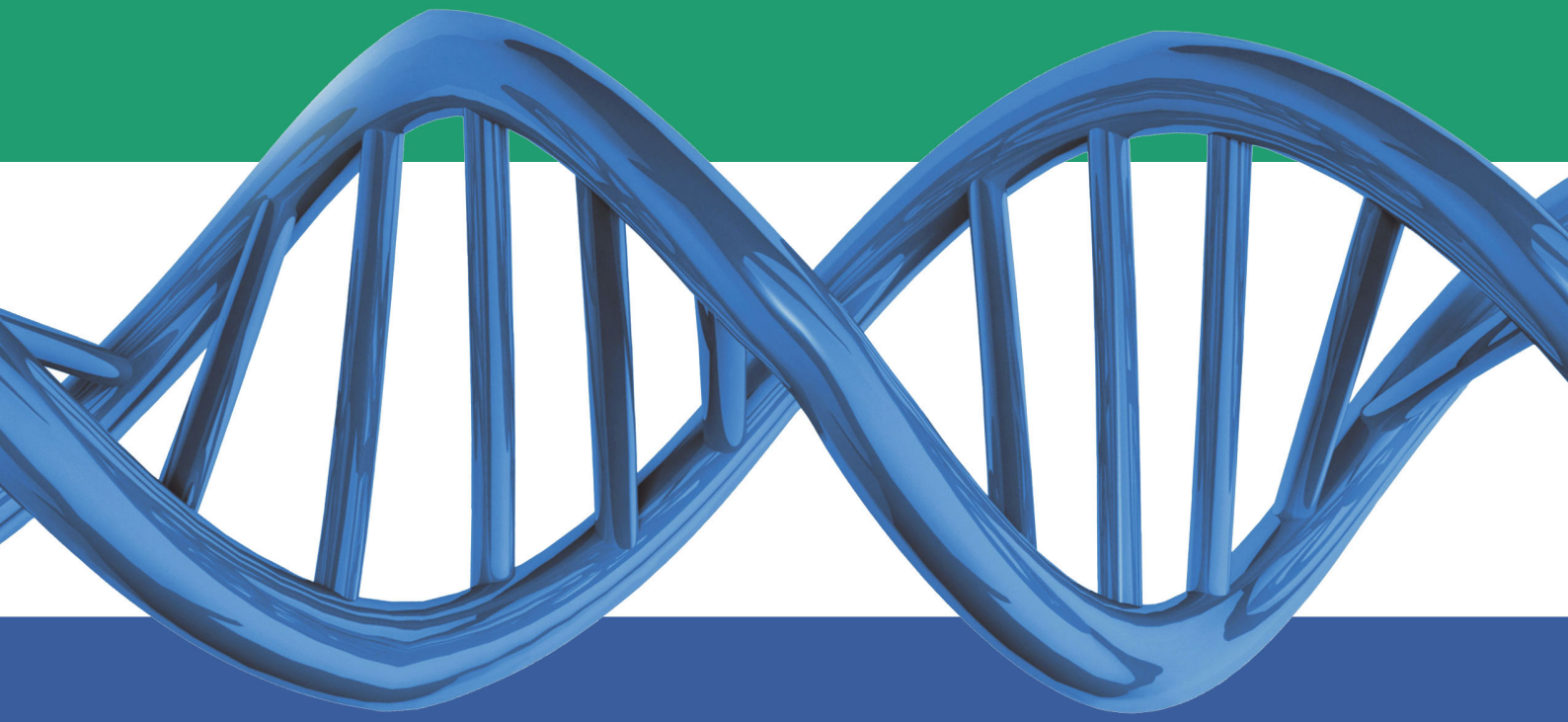
ISBN: 978-1-68108-024-6

e-ISBN: 978-1-68108-023-9

Advances in Modern Medicine



Kiyomi Taniyama
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Advances in Modern Medicine

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Advances in Modern Medicine

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ISBN (eBook): 978-1-68108-023-9

ISBN (Print): 978-1-68108-024-6

First published in 2017.

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FOREWORDS

FOREWORD 1

CONGRATULATION ON THE PUBLICATION OF "ADVANCES IN MODERN MEDICINE"

Hideo Kusuoka

We congratulate Dr. Kiyomi Taniyama and Dr. Wataru Kamiike on the publication of this excellent eBook entitled "*Advances in Modern Medicine*." This publication is the outcome of more than ten years of effort at the Institute for Clinical Research, Kure Medical Center and Chugoku Cancer Center (KMCCCC), one of the major hospitals in the National Hospital Organization (NHO).

NHO is an independent administrative agency that manages 143 hospitals nationwide in Japan. The NHO is entrusted with three missions: providing medical care, promoting clinical research and educating medical care professionals. We are the largest domestic hospital network, providing services from acute to chronic care. There are more than 80 clinical research institutes attached to the hospitals. The Institute for Clinical Research of KMCCCC is one of the most powerful research institutes within NHO.

It has been pointed out recently that the clinical research should be more strenuously promoted in Japan, because domestically clinical research is not as active compared to basic medical research. As such, we are promoting high-quality clinical research including clinical trials with rapid progress by leveraging NHO's large network. Looking forward, we expect continuous growth at the Institute for Clinical Research of KMCCCC, and hope that KMCCCC will be a leader for clinical research within NHO and also throughout Japan.

Hideo Kusuoka
President, National Hospital Organization,
Tokyo, Japan

FOREWORD 2

TOWARD ENHANCEMENT OF RESEARCH ACTIVITIES IN CLINICAL MEDICINE

Takaaki Kirino

This publication was planned to celebrate the 10-year anniversary of the Institute for Clinical Research, Kure Medical Center and Chugoku Cancer Center (KMCCCC). Dr. Kiyomi Taniyama has served as the director of this institute throughout these 10 years. In addition, the publication is intended to commemorate the contributions and dedication of Dr. Wataru Kamiike, the president of KMCCCC, who will retire from his position in July.

The city of Kure, home to Kure Medical Center, used to be one of Japan's major naval bases. After World War II, operation of the Kure Naval Hospital was taken over by the United Nations. The hospital was transferred to the Japanese government 10 years later and began to serve the Japanese people as a national hospital. Since then, KMCCCC has stood as the leading hospital in the Chugoku-Shikoku region. The role of the center has not been limited to clinical services for people in the Kure area, however. The center has also been expected to enhance and advance research activities in clinical medicine. The Institute for Clinical Research, KMCCCC was established and has functioned for this purpose.

Japan is generally acknowledged as a nation with the highest standards and achievements in scientific research. This is true for basic research such as research to elucidate the molecular mechanism of diseases. However, Japan's achievements in clinical investigation are somewhat limited. International rankings of research activities have listed Japan 3rd in the field of basic medical sciences, but 18th in clinical medicine.

The National Hospital Organization is expected to encourage each member hospital to enhance its research activities in clinical medicine, including case reports, case-controlled studies, clinical trials, and cohort studies. Progress in medicine has been accomplished only through careful observation of clinical cases. Evidence that is inevitably needed for daily clinical decision making is only possible through documentation of clinical experiences.

We anticipate that the strength of research at the Institute for Clinical Research, KMCCCC, led by Dr. Taniyama, will continue to grow; it is our hope that the institute will contribute significant advancements to the medical sciences well into the future.

Takaaki Kirino
Former President, National Hospital Organization,
Tokyo, Japan

FOREWORD 3

10 GLORIOUS YEARS FOR THE NATIONAL HOSPITAL ORGANIZATION KURE MEDICAL CENTER AND CHUGOKU CANCER CENTER

Fumitaka Saji

I want to express my great appreciation to Dr. Wataru Kamiike, president of national hospital organization (NHO) Kure Medical Center and Chugoku Cancer Center (KMCCCC), for his 10 years of significant contributions to the hospital. In his first five years, he helped me in his role as vice president to reengineer hospital management and in the second five years he managed the hospital as its president. Over the past decade, KMCCCC has made remarkable progress in both clinical and financial performance, which resulted in the hospital being acknowledged as one of the best hospitals among the more than 140 hospitals within the NHO. I believe that our highly advanced medical treatment and diligent nursing by the hospital staff have produced these outstanding results.

Publication of this eBook titled "Advances in modern medicine during the last decade" upon Dr. Kamiike's retirement and the tenth anniversary of Dr. Taniyama as director of the Clinical Research Institute affiliated with KMCCCC is extremely timely.

Dr. Taniyama will serve as the next president of KMCCCC. He has also contributed to the hospital's distinguished development in both basic and clinical research. He is a highly regarded scientist in the field of clinical pathology not only domestically but also internationally. The memorandum of understanding between KMCCCC and Rajavithi Hospital in Thailand, the Kure International Medical Forum, and other international activities could not have been a great successes without his effort.

I thank everyone who has contributed to this eBook. I hope its publication will be a key milestone for the KMCCCC and that the center continues to grow and contribute to the advancement of modern medicine.

Fumitaka Saji
Honorary President, NHO Kure Medical Center & Chugoku Cancer Center,
Kure, Japan

&

CEO, Ashiya Municipal Hospital
Ashiya, Japan

FOREWORD 4

CONGRATULATORY MESSAGE FOR DR. KIYOMI TANIYAMA, KURE MEDICAL CENTER

Wataru Yasui

It is my great honor and pleasure to write this congratulatory message for Dr. Kiyomi Taniyama on the occasion of the publication of his eBook, *Advances in Modern Medicine during the Last Decade* which coincides with the tenth anniversary of his chairmanship at the Institute for Clinical Research and his appointment as the President of National Hospital Organization Kure Medical Center and Chugoku Cancer Center (KMCCCC).

This eBook has taken up basic science, translational research and modern medicine in relation to both diagnosis and treatment, and reviews the latest cutting-edge therapeutics and diagnostics by KMCCCC's entire staff. I am deeply impressed by the eBook's descriptions of the achievements of the last decade. There is no doubt that this eBook is valuable to medical workers and researchers around the world.

I have known Dr. Taniyama for more than 30 years, since I became a member of the First Department of Pathology at Hiroshima University. Over the years, I have learned much, not only about medicine and pathology but also about social issues. I admire his foresight and action. In addition to his pathology practice and research, he organizes the annual Kure International Medical Forum and collaborates with many medical professionals across Asia. He is a leader and makes the resources of the Pathology Clinic available to cancer patients in Japan. When I, as the President, hosted the 103rd Annual Meeting the Japanese Society of Pathology in April 2014, he chaired the program committee of diagnostic pathology and ensured the great success of the meeting.

I wish Dr. Taniyama and his colleagues in KMCCCC all the best for a bright future. As the Director of Institute of Biomedical & Health Sciences, Hiroshima University, I look forward to our collaboration to achieve our goal of an even better understanding and treatment of our patients and the improvement of human health worldwide.

Wataru Yasui
Professor and Chairman, Department of Molecular Pathology
Director, Institute of Biomedical & Health Sciences
Dean, Hiroshima University Institute of Biomedical & Health Sciences,
Hiroshima, Japan

PREFACE

Kiyomi Taniyama

Wataru Kamiike

The National Hospital Organization (NHO) of Japan was rebuilt from the former National Hospitals of Japan 10 years ago in 2004. At almost the same time, Dr. Kiyomi Taniyama obtained his position as the director of the clinical research institute affiliated with the NHO Kure Medical Center and Chugoku Cancer Center (KMCCCC), Kure, Japan. Over the last decade, he has organized his research institute well and obtained many good results with the collaboration of the basic and translational research staff. Because the systems and equipment are often upgraded to keep pace with the latest trends in medicine, remarkable medical advances have been achieved during the last decade in our hospital, which is representative of the progress made in modern medicine, not only in Japan, but also around the world.

On June 30, 2014, Dr. Wataru Kamiike, is going to retire from the president of NHOKMCCCC that is one of the top five hospitals among the 143 NHO hospitals in Japan. Therefore, July in 2014 is a good time to celebrate Dr. Taniyama's ten-year history as the director of clinical research center and promotion to the next president, as well as the retirement of Dr. Kamiike from the president. Fundamentally, this eBook will be a memorable token of this celebration, but it also represents the latest trends in modern medicine, covering a wide range of fields. As previously mentioned, this eBook will cover basic science, translational research, and modern medicine in relation to both diagnosis and treatment. This eBook will be full of reviews of cutting edge therapies and diagnostic techniques related to the fields of the authors, along with original articles with the latest data obtained by the authors.

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Part 1
TOPICS IN THE MODERN MEDICINE
A: CANCER MANAGEMENT

CHAPTER 1

A New Therapeutic Strategy for Advanced Squamous Cell Carcinomas of the Head and Neck

Haruo Hirakawa[1,*], Yasuyuki Nishi[1], Taisuke Watanabe[1], Makoto Tada[1], Kiyomi Taniyama[2] and **Wataru Kamiike[2]**

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[2] *National Hospital Organization Kure Medical Center and Chugoku Cancer Center, Kure, Japan*

Abstract: A concurrent superselective intra-arterial chemoradiotherapy (SIACRT) is a new therapeutic strategy for advanced squamous cell carcinomas of the maxillary sinus and upper gingiva that might enable patients to keep the shape and function as well as improve curability. We have been applying SIACRT since 2008. We will present the results of SIACRT compared to conventional combined therapy at our hospital. SIACRT is promising to preserve patient's quality of life (QOL) both under and after the treatment without sacrificing a cure rate.

Keywords: Cisplatin, Head and neck cancer, Superselective intra-arterial chemoradiotherapy, 5-Fluorouracil.

INTRODUCTION

A combination of intra-arterial chemotherapy, radiotherapy (RT), and surgical therapy has been a common therapy for advanced squamous cell carcinomas (SCC) of the maxillary sinus and upper gingiva in Japan for nearly 30 years [1]. The conventional combined therapy (CTx) has remarkably improved the survival rate compared to each therapy alone; however, functional and cosmetic problems remain. The longer patients survived after treatment, the more they suffered from

[*] **Corresponding author Haruo Hirakawa:** Department of Otorhinolaryngology, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, Hiroshima, Japan; Tel: +81-82-241-3111; Fax: +81-82-246-0676; E-mail: hirakawah@hiroshima-med.jrc.or.jp

Kiyomi Taniyama & Wataru Kamiike (Eds.)

face deformity and dysfunctions of speech, mastication, and vision, even though they were free of the disease.

A concurrent chemoradiotherapy (CRT) has become a standard therapy for advanced pharyngeal and laryngeal SCC, which enables patients to keep the functions of the larynx and pharynx (*i.e.*, speaking and swallowing) without sacrificing curability [2].

Superselective intra-arterial chemotherapy (SIAC) has more effect on the target lesion without increasing general adverse effect than general chemotherapy. Localized cancer without metastatic lesion is good indication for SIAC [3].

SIACRT, combination of CRT and SIAC, is a new therapeutic strategy for advanced squamous cell carcinomas of the maxillary sinus and upper gingiva that might enable patients to keep the shape and function as well as improve curability [4]. We have been applying SIACRT since 2008. We present the results of SIACRT compared to CTx at our hospital.

PATIENTS AND METHODS

CTx was applied to 9 cases from 2004 to 2010, containing 6 males and 3 females of which the age-range was from 61 to 80.

SIACRT was applied to 10 cases from 2009 to 2012, containing 7 males and 3 females of which the age-range was 57 to 81.

A carcinoma of the upper gingiva involving the maxillary sinus was treated in the same way as a maxillary cancer involving the upper gingiva.

PROTOCOL OF CTX (FIG. 1)

The 1st day, the affected side of the maxillary sinus is opened through transalveolar incision to reduce the volume of the tumor and to examine the frozen section histopathologically if necessary. An indwelling catheter is inserted into the superficial temporal artery under local anesthesia.

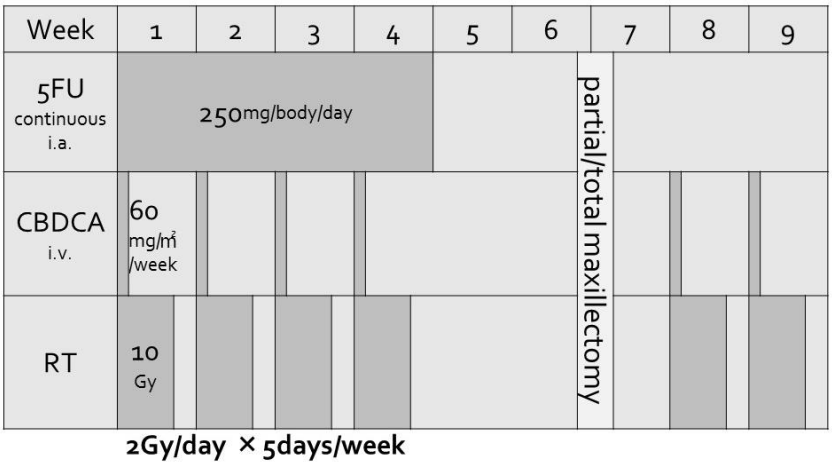

Fig. (1). Protocol of combined therapy. It takes nine weeks to complete.

The 2nd day, irradiation, continuous intra-arterial administration of 5 fluorouracil (5FU), and intra-venous weekly administration of carboplatin (CBDCA) are started. Daily necrotomy of the tumor is performed with the maxillary sinus open till the surgery. Partial or total maxillectomy is performed 2 weeks after a total dose of 40Gy irradiation. A total dose of 10 to 20Gy additional irradiation and weekly administration of CBDCA are started again 1 week after the surgery.

PROTOCOL OF SIACRT (FIG. 2)

Intra-arterial micro-catheter is inserted into a feeding artery of the tumor super-selectively with Seldinger's method contrasting the tumor. Cisplatin (CDDP) is intra-arterially injected into the tumor through the catheter at the rate of 5 mg a minute. At the same time sodium thiosulfate (STS) was intravenously administered at 200-fold the dose of CDDP in molar quantity to reduce the toxicity of CDDP. Irradiation was started on the same day. Five-day-continuous intra-venous administration of 5FU was started on the next day. CDDP and 5Fu were administered in the same way four weeks later. A total dose of irradiation was 50 to 60 Gy.

CHAPTER 2

The Long-Term Outcomes of Partial Breast Irradiation Using External Beam after Breast Conserving Surgery

Michinori Yamamoto*

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Abstract: To examine long-term outcomes of a single institution's cases of partial breast irradiation.

Between January 1990 and March 2001, a total of 104 patients with T1 or T2 breast cancer were treated with partial breast irradiation using external beam after breast-conserving surgery. Ipsilateral breast tumor recurrence (IBTR) and contralateral breast tumor occurrence (CBTO) were examined. Median follow-up time was 10.2 years.

IBTR rates at 5, 10 and 15 years were 2%, 6.5% and 13.2%, respectively. CBTO rates at 5, 10 and 15 years were 0%, 1% and 5%, respectively. Failure within radiation field occurred in 1 patient and failure outside radiation field occurred in 7 patients. Of 7 recurrences outside radiation field, 1 recurred diffusely in the skin of the breast, 3 recurred in another portion of the breast and 3 recurred in the breast near the radiation field. The incidence of failure outside radiation field was high compared to that of CBTO, but this difference was not statistically significant (p=0.059).

This study speculates that PBI is a feasible alternative to WBI.

Keywords: Breast cancer, External beam irradiation, Partial breast irradiation, Recurrence, Surgery.

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INTRODUCTION

Breast-conserving therapy is an effective treatment method for T1 or T2 breast cancer [1]. In the standard treatment protocol, the surgeon resects a clinically detected tumor and microscopic residual diseases is then eradicated with whole breast irradiation (WBI), which requires a comparatively long treatment period of about five or six weeks. Treatment could be made more convenient for the patient by the adoption of accelerated partial breast irradiation (APBI), which requires only five or six days; this method has been tried and promising results have been published [2 - 8]. When the radiation field is restricted to the tumor bed, however, there is a risk of ipsilateral breast tumor recurrence (IBTR) outside the radiation field. Holland *et al.* have studied the multifocality of breast cancer in mastectomy specimens and have reported that 11% to 18% of patients would have had microscopic residual diseases if the primary tumor had been removed with a margin of only 3 to 4 cm [9]. Since breast cancer usually grows slowly, long follow-up was necessary to confirm the hypothesis that APBI is a feasible alternative to WBI. We started partial breast irradiation (PBI) using external beam in 1990; the median follow-up time for these cases is 10.2 years (range, 1 to 221 months). Here, we present the long-term outcomes of our PBI cases and evaluate a risk of IBTR outside the radiation field.

MATERIALS AND METHODS

Patients and Tumor Characteristics

Between January 1990 and March 2001, 109 patients with T1 or T2 breast cancer were treated with PBI after breast-conserving surgery. Of 109 patients, 5 were excluded from this study, since they were lost to follow-up within 5 years (7, 12, 31, 45 and 49 months). The remaining 104 patients' ages ranged from 30 to 79 years (median: 54 years). Fifty-four lesions were in the right breast and 50 lesions were in the left. Each patient underwent partial resection or quadrantectomy of the breast, the margins of which were negative except in two cases. Only 4 cases were invasive lobular carcinoma, which had a higher frequency of multifocality than invasive ductal carcinoma did. All patients except one also underwent axillary dissection. The pathological TNM-classification of the tumors, (UICC, 2002), are

shown in Table **1**.

Table 1. Pathologicl TNM-classification of tumors examined.

Lymph Node Metastasis	T1a	T1b	T1c	T2
N0	1	3	65	17
N1a	0	1	12	4
N2a	0	0	1	0

UICC, 2002

Treatment

The remaining breast was subjected to radiation therapy with electron beam (EB) except in two cases. The EB radiation fields were determined by a radiation oncologist and a surgeon based on the location and extent of the surgical scar and all available clinical records. EB field sizes ranged between 5 x 6 cm^2 and 11 x 13 cm^2; the most popular field size was 6 x 10 cm^2. The EB energies used were 5MeV in 2 cases; 7MeV in 22 cases; 9MeV in 43 cases; 11MeV in 31 cases and 13MeV in 4 cases. The remaining 2 cases, in which the tumors were located in the upper outer quadrant of the breast, were treated with Cobalt-60, while the upper outer quadrant of the remaining breast as well as the axillary and supraclavicular regions were irradiated in an anterior photon beam. Delivered doses ranged between 44 and 52 Gy in 15-25 daily fractions over five weeks in patients with negative margins and 60 and 70 Gy in 30 and 35 daily fractions over six and seven weeks in 2 patients with positive margins. In cases of inpatient treatments, radiation therapy was delivered in daily fractions of 2 Gy, 5 times per week; in cases of outpatient treatments, it was delivered in daily fractions of 2.5 or 3 Gy, 3 times per week, for the sake of the patients' convenience. Of 104 patients, 89 (86%) were treated with daily fractions of 2 Gy, 5 times per week, to a total dose of 50 Gy.

Four patients also received adjuvant systemic chemotherapy, 79 patients received adjuvant systemic endocrine therapy, and 11 patients received both.

End Points and Statistics

The endpoints analyzed were ipsilateral breast tumor recurrence (IBTR),

CHAPTER 3

Is It Possible to Optimize Neoadjuvant Chemotherapy Response by EGFR and CK5/6 Expression Status in Breast Cancer Patients?

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Abstract: We examined the expression of ER, HER2, Ki-67, CK5/6 and EGFR by immunohistochemistry in breast tumors from patients who underwent neoadjuvant chemotherapy (NAC).

88 breast cancer patients who received NAC and surgery at our institute between January 2008 and March 2013 were enrolled. Staining results of ER, HER2, CK5/6, EGFR and Ki-67 were quantitated by automated immunostaining analysis. Patients were stratified into four grades (0, 1+, 2+, 3+) by ER status. Ki-67 index and nuclear grade were compared between a CK5/6- and/or EGFR-positive cohort and a CK5/6- and EGFR-negative cohort for each ER status grade. We also assessed response to chemotherapy according to ER, CK5/6 and EGFR status in a HER2 negative cohort.

The percentage of CK5/6- and/or EGFR-positive tumors decreased inversely with increasing degree of ER expression. In the ER0 cohort, the CK5/6- and/or EGFR-positive cohort had a higher Ki-67 index (p=.0875) and nuclear Grade 3 (p=.0036) than a CK5/6- and EGFR-negative cohort. A CK5/6- and/or EGFR-positive cohort showed a higher tumor reduction rate of clinical effect than a CK5/6- and EGFR-negative cohort

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(mean=57.3% and 24.6%, respectively, p=.2053) in an ER0 cohort. In the CK5/6- and/or EGFR-positive cohort, two of nine showed Grade 3 and seven showed Grade 2a or more of pathological effect. In cohorts of ER2+ or ER3+, there was no correlation between CK5/6 and EGFR status and response to chemotherapy.

In triple negative breast cancer (TNBC), CK5/6 and EGFR expression can be utilized as markers that differentiate the effect of NAC.

Keywords: CK5/6, EGFR, Estrogen receptor, Ki-67, Triple negative breast cancer.

INTRODUCTION

In Japan, the current incidence of newly diagnosed primary breast cancer is 50,000 per year. Cancer screening, progress in diagnostic technology, and treatment have contributed to an improved five-year survival rate that is estimated at 90.7%. Initial treatment for primary breast cancer is standardized internationally by National Comprehensive Cancer Network (NCCN) guidelines (http://www.nccn.org) and the St. Gallen consensus [1], which is based on an understanding of the molecular biological characteristics of breast cancer. Molecular biological characteristics are classified into some subtypes based on breast cancer gene clusters [2]. In common clinical settings, we use immunohistochemical classification that is determined by the expression status of ER, PR, HER2 [3] and proliferative markers such as Ki-67 [4], instead of histological subtypes. It has been confirmed that basal-like breast cancer has an aggressive character and poor prognosis. It expresses CK5/6 and EGFR in gene analysis. There have been several approaches to use of immunohistochemical expression status of CK5/6 and EGFR as a surrogate for gene clustering. However, staining protocol, criteria, and clinical impact of CK5/6 and EGFR remain to be established. The aim of this study is to clarify the impact of CK5/6 and EGFR expression in a clinical setting.

MATERIALS AND METHODS

Patients

A total of 108 consecutive patients who received neoadjuvant chemotherapy (NAC) and surgery at our institute during the period between January 2008 and March 2013 were enrolled in the present study. We received informed consent from each patient with his/her signature and the ethics committees of Kure Medical Center and Chugoku Cancer Center approved the study design.

Immunohistochemistry

Prior to the chemotherapy, we obtained specimens by core needle biopsy from all patients. Immunohistochemistry (IHC) for ER, PR, HER2, EGFR, CK5/6 and Ki-67 was performed using a Benchmark XT automated stainer (Roche, Basel, Switzerland) according to the manufacturer's instructions with some modifications as reported previously [5]. ER (1D5, Dako), PR (PgR636, Dako) and Ki-67(MIB-1, Dako) antibodies were used at 1:50 dilution, respectively. HER2 (4B5, Roche) antibody was used at 1:1 dilution. CK5/6 (D5/16B4, Dako) antibody was used at 1:100 dilution. Antigen-antibody complexes were detected with an I-VIEWDAB detection system (Roche) on the Benchmark XT. Immunohistochemistry for EGFR was performed using the EGFR pharmDX^{TM}kit (Dako) on an Autostainer (Dako). Staining results of each marker were quantitated with automated immunostaining analysis software (Genie, Leica Microsystems, Germany) [5, 6]. Analysis of fluorescent *in situ* hybridization (FISH) for the HER2 gene was done using a PathVysion kit (Abbot, Tokyo, Japan).

ER, PR and Ki-67 were considered to be positive if immunostaining was seen within the nuclei of invasive carcinoma cells. ER, PR and Ki-67 results were reported as a percentage of positive cells. The prevalence of ER and PR were scored according to a partially-modified J-score scoring system [7], where positive cells were <1% (0); 1% to 10% (1); 10% to 50% (2); and 50% or more (3) of tumor cells examined. Regarding Ki-67 status, five fields with higher Ki-67 labelling at 50,000 μm^2 per field were selected and mean of the prevalence of Ki-67 in five fields was defined as the Ki-67 index [5]. HER2 was defined to be

CHAPTER 4

Auto-Analysis of Immunohistochemical Findings for Breast Cancer

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Abstract: Immunohistochemical (IHC) and molecular findings with their histopathological diagnosis are critical in the current therapeutic strategy for a breast cancer patient. These findings require considerable manual human involvement and interpretations can be subjective. In this study, we examined the usefulness of an auto-analysis computer system for analysis of IHC findings.

Forty breast-cancer specimens were examined for IHC expressions of estrogen receptor (ER), progesterone receptor (PgR), HER2, Ki-67 and Topoisomerase II alpha (TopoIIa). One-hundred-fifty-six cancer specimens were examined for HER2. Histopathological images of IHC specimens were stored digitally thru virtual microscopy (Hamamatsu Photonics). A Genie/Aperio software package on a desktop computer auto-analyzed these images.

For ER and PgR, concordant results were obtained between ocular observation and auto-analysis ($p < 0.001$). The Ki-67 index ($r = 0.96$) and TopoIIa index ($r = 0.95$) also showed a significant correlation ($p < 0.001$). Concordant ratios were 92.9%, 82.2%,

59.5% and 100% for HER2 score 0, 1, 2 and 3, respectively. 24 cases (28.6%) of 84 cases with a HER2 score of 2 by ocular observation were re-analyzed as a score of 3 by

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auto-analysis. 19 cases (79.2%) of these re-scored cases showed HER2 gene amplification by FISH analysis.

In sum, well-organized auto-analysis is able to provide objective results. Thus, auto-analysis might be a means by which to standardize methods for immunohistochemical detection of breast cancer.

Keywords: Auto analysis, Breast cancer, Immunohistochemistry, Ocular observation, Virtual microscopy.

INTRODUCTION

Immunohistochemical (IHC) and molecular findings of an estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor 2 (HER2), Ki-67, and Topoisomerase II alpha (TopoIIa) are important in determinations about hormonotherapy or chemotherapy in current breast cancer therapy [1, 2]. Due to the considerable involvement of pathologists or medical technologists to obtain these findings, there are limitations due to intra-observer error and potentially subjective decision-making. Recently, computerized auto-image analysis was developed. There are only a few reports regarding its usefulness in breast cancer therapy [3 - 5]. As such, in this study, building upon our past report [4], we further examined the usefulness of Genie/Aperio auto-analysis computer software.

MATERIALS AND METHODS

40 breast cancer specimens (25 biopsy materials and 15 surgically resected materials) were collected from November 2009 to March 2010 and from March to April 2013 and were examined for IHC expressions of ER, PgR, HER2, Ki-67, and TopoIIa. The average age of patients who have these breast cancers was 60.1 years old. One-hundred-fifty-six cancer specimens (72 biopsy materials and 84 surgically resected materials) collected from April 2002 to October 2010 and from March to April 2013 were examined for HER2. The average age of these patients was 58.8 years old. Specimen preparation and image analysis were performed as previously described [3, 4]. For surgically resected specimens, small pieces for IHC and FISH analysis were immediately cut from the materials. Biopsy and surgically resected specimens were fixed in 10% neutral formalin buffer for more

than 12 hours, but less than 72 hours. Paraffin embedded specimens were cut 3 to 4 μm thickness. Seven pieces per 1 specimen (HE 1, IHC 5, and FISH 1) were made. Immunostaining was performed using a labeled streptavidin biotinylated-peroxidase (LSAB) method. The primary antibodies used were ER (1D5, Dako, 1:50; SP1, Ventana, 1:1), PgR (PgR636, Dako, 1:50; 1E2, Ventana, 1:1), HER2 (erb2, Dako, 1:1; 4B5, Roche, 1:1), Ki-67 (MIB1, Dako, 1:50) and TopoIIa (Ki-S1, Dako, 1:800). IHC staining was performed using auto-stainers Benchmark XT (Roche, Basel, Schweiz) according to the manufactory's protocol. Cultured cells (Pathology Institute, Toyama, Japan) were used as control specimens. FISH of the HER2 gene was performed using PathVysion (Abbot, Tokyo, Japan).

For evaluation of ER, PgR and HER2, we examined the whole field of a specimen except for intraductal carcinoma components. An ER and PgR positive rate was classified as score 0 (< 1%), score 1 (1 - 10%), score 2 (10 - 50%) and score 3 (50% <). HER2 expression was evaluated according to guidelines by the American Society of Clinical Oncology (ASCO) and The Japanese Breast Cancer Society [6, 7]. The index for Ki-67 and TopoIIa was defined as average percentage of positive cells in 5 high power fields (x40, 1 field area: about 72,000 μm^2) as previously described [3, 4]. We performed auto-analysis using virtual microscopy (VM) (Hamamatsu Photonics, Hamamatsu, Japan) and Genie/Aperio (Vista, CA, USA) computer software as previously described [3, 4]. Genie is software that can identify tumor cells from non-tumor cells and stromal tissue with higher precision by learning certain types of histology. Identified tumor cells were analyzed by image analysis algorithms for cell nuclei (Nuclear v9.1) or membranes (Membrane v9.1). Results from ocular observation and auto-analysis were compared. HER2 gene copy status was examined by FISH [8]. The use of samples for this study was approved by the Institutional Ethical Board at the Kure Medical Center and Chugoku Cancer Center (NHO-KureH141129).

STATISTICAL ANALYSIS

Comparison was performed using JMP for Windows (version 9.0) statistical software package (SAS Institute, NG). The results of patients' age were expressed as means. Concordant results of ER, PgR, Ki-67 and TopoIIa between ocular observation and auto-analysis were obtained by Pearson product-moment

CHAPTER 5

How does Pathology Clinic Have Effect on Mental State and Adjustment in Patients with Breast Cancer?

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Abstract: We conducted a paper-based questionnaire and interview targeting patients with breast cancer who had undergone radical surgery, before and after they attended the Pathology Clinic, to investigate the short-term impact on the psychological state and attitude of the patients from attending the Pathology Clinic. There may be increased motivation for treatment and a sense of reassurance, as well as reduction in anxiety in patients as a status indicated by HADS in the group that attended the Pathology Clinic, so this suggests that attending the Pathology Clinic may reduce anxiety in a short term. On the other hand, the MAC Anxious Preoccupation score was significantly higher in the group that attended the Pathology Clinic, both before and after attendance, than the group that did not attend. Therefore, the group that attended may have had characteristics of eliminating anxiety by proactive actions including collecting medical data on the cause of anxiety and adopting healthy behaviors. The result suggests that appropriate support in terms of medical information and emotion is necessary.

Keywords: Breast cancer, HADS, MAC, Mental state, Pathology clinic.

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INTRODUCTION

Cancer has been the leading cause of death since 1980 and it has long been said that cancer will affect 1 in every 2 to 3 people in Japan. Currently, cancer treatment has advanced remarkably, a wide variety of treatment options are available, and the perception that cancer should be regarded as a "chronic disease", much like diabetes and kidney disease, has become common. Therefore, the intention of treatment now includes not only prolonging life, but also improving and maintaining the quality of life (QOL) of patients with cancer. As a prerequisite for this, it is essential to disclose information on cancer pathology and treatment methods, and patients themselves have come to demand accurate information on their own diseases. In the current era a plethora of tools that enable instantaneous acquisition of a wide variety of information have evolved. While this has made information easy to obtain, the reality is that various medical information significantly impacts upon the anxiety, mental state and psychological adjustment of patients.

The name of Diagnostic Pathology was allowed by Japanese Government to be advertised at the hospital in April 2008, necessitating the pathologists to promptly record data in medical records on pathological diagnosis and cytodiagnosis, instruct patients or their guardians on treatment methods and other such matters, and respond to requests from patients for direct explanations [6]. In this center a Pathology Clinic has been established by pathologists in 2006 before the Diagnostic Pathology department has been established. In the Pathology Clinic the results of the pathological diagnosis (diagnosis obtained through observation of the collected pathological tissue and cells) is explained in detail by pathologists to patients who wish to know this information. Since many of the patients and their families consider that cancer will be cured if they undergo a surgery, they are confused sometimes by treatment continuing after surgery. In addition to an explanation of the disease state and treatment options by the attending physician, an explanation of the pathology results in the Pathology Clinic provides the necessary and accurate information to the patients themselves, thereby promoting the patients' understanding of the disease and basis of adjuvant therapy chosen. This is expected to change the mental state, recognition and behavior towards both the disease and treatment, and in fact, clinical cases manifesting these

changes have been reported [7].

Therefore, in this study, to clarify the short-term effect of the Pathology Clinic, we investigated the changes in the patient's mental state and psychological adjustment to cancer between 2 time points with an approximately one month interval, *i.e.*, one after surgery and the other immediately after attending the Pathology Clinic, in breast cancer patients who had undergone radical breast cancer surgery. We simultaneously conducted a comparative study in these patients *versus* a patient group that had not attended the Pathology Clinic.

METHODS

Subjects

Of the 215 female patients with breast cancer who had undergone radical breast cancer surgery between July 2008 and December 2010, and were recommended to attend the Pathology Clinic after surgery by their attending breast cancer surgeons, we surveyed 55 patients who consented to participate in the survey. The 55 patients who consented to the survey were surveyed with a total of 2 times, one between surgery and discharge from the hospital (pretest) and the other immediately after attending the Pathology Clinic (posttest). Questionnaire data analysis included 34 of these patients who completed both questionnaires (attendance group n = 29, non-attendance group n=5). In addition to the questionnaire survey, in the post test the patients were interviewed, and 12 of the 34 patients consented to this interview (attendance group n=11, non-attendance group n=1).

Pathology Clinic Procedures and Details

Subjects were handed a pamphlet on the Pathology Clinic at admission by the attending breast cancer surgeon. A psychotherapist visited the subject individually during their hospital stay and asked whether they wished to attend the Pathology Clinic. If the subject expressed a desire to attend the Pathology Clinic, the psychotherapist submitted a request to the pathologist and the subject attended the Pathology Clinic approximately 1 month after surgery.

CHAPTER 6

Recurrence Pattern and Long-Term Survival After Two Types of VATS Lobectomy for Clinical T1N0 Lung Cancer

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Abstract: To provide a less invasive procedure in video-assisted thoracic surgery (VATS), it is necessary to evaluate two types of VATS approaches in terms of clinical outcome, especially cancer recurrence.

We conducted a prospective feasibility study of two strictly defined types of VATS lobectomy by prospective cohort. Based on a patients' decision after a similar preoperative explanation, cancer recurrence and long-term-prognosis were evaluated by comparing among assisted VATS and complete VATS groups.

One-hundred-four consecutive patients with clinical T1N0M0 non-small cell lung cancer (NSCLC) were analyzed. Twenty-six cases (ASSIST group) chose lobectomy performed through an anterolateral small thoracotomy with the use of a rib spreader in combination with a thoracoscopic view and direct view. Seventy-eight cases (PURE group) chose complete VATS in which only a monitor was used during smaller access thoracotomy without a rib spreader. Patients in the PURE group exhibited early recovery from surgery. The recurrence rate was equivalent (both 19.2%). At 2,039 days of a median follow-up period, the recurrence pattern was also similar between the two groups; however, two cases (2.6%) of pleural dissemination appeared only in the PURE group. Specific clinicopathological characteristics were not confirmed in the recurrent cases. Recurrence-free and overall 5-year survival rates were equivalent between the two groups.

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Both PURE and ASSIST are feasible in terms of cancer recurrence and long-term prognosis.

Keywords: Lobectomy, Minimally invasive surgery, Non-small cell lung cancer, Recurrence rate, Video-assisted thoracic surgery.

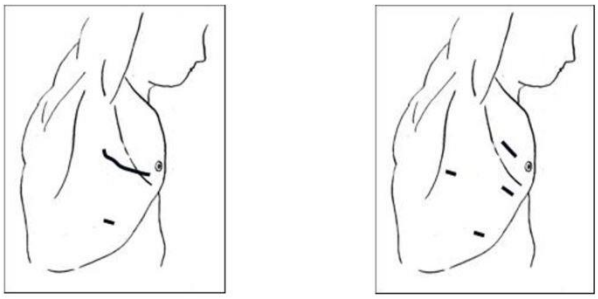
INTRODUCTION

Non-invasive procedures in endoscopic surgery have contributed to minimizing operative stress. Lobectomy with lymphadenectomy for lung cancer is known as video-assisted thoracic surgery (VATS). There remained, however, many unresolved issues, especially regarding the influence on recurrence, and prognosis from an oncological aspect. Particularly, the technical approaches of a VATS lobectomy varies in the literature [1] based on facility and surgeon [2], which seriously confounds our evaluation of this promising approach. Therefore, a VATS lobectomy is not currently regarded as a standard operative procedure for lung cancer because of a lack of confirmative evidence. However, it would be extremely difficult to conduct a randomized comparative study because VATS is already regarded as the usual clinical procedure.

Our aim was to assess cancer recurrence, and prognosis among two VATS methods, in which indication was prospectively determined by a patient's decision.

Lung carcinomas were diagnosed as clinical T1N0M0 non-small cell lung carcinoma (NSCLC) by high-resolution CT scan in all cases. Cases were diagnosed by preoperative biopsy through fiberscope or simultaneous VATS. The seventh edition *International Union against Cancer* (Union for International Cancer Control; UICC) on Cancer TNM classification was applied to all cases. To measure patient comorbidities, the Charlson Comorbidity Index was applied to all patients [3]. The distinction between the two methods was explained uniformly by either of two regular surgeons who received the same patients. Selection was completely based on patients' decisions before surgery. Surgery was performed under general anesthesia with separation of lung ventilation by either of the two regular surgeons in a single institute according to the study schema (Fig. **1**). The aim of this study was to provide a prospective evaluation of the differences in

recurrence pattern among the two types of VATS lobectomy.



a b

	Rib spreader	Skin incision	Cutting length of intercostal muscle	How to use thoracoscope
ASSIST	+	**4-10 cm**	much longer than skin incision	light source>>monitor
PURE	–	**3 cm**	Same as skin incision	monitor

Fig. (1). Baseline characteristics of two VATS approaches.
a. Assist VATS lobectomy (ASSIST) **b.** Pure VATS lobectomy (PURE)

Written informed consent was obtained from each patient and institutional review board approval was obtained to collect data from a secure database.

Procedures for each type of VATS lobectomy has been previously defined in detail [4, 5]. We preferably used a flexible thoracoscope (Olympus, Japan) as monitoring in PURE or as a light guide in ASSIST. The techniques in the ASSIST and PURE groups were performed in the same manner except for the approach and the operator's eye direction. A pair of bipolar scissors (Bissinger, Germany) was held in the operator's right hand when dissecting the tissue through the minithoracotomy wound in the same manner for the two groups (Fig. **2**). In both groups, the upper mediastinal nodes were similarly dissected in cases of upper lobe carcinoma and the subcarinal nodes were dissected in the cases of lower lobe carcinoma. Both lymph node sites were additionally dissected in case of middle lobe cancer and the node-positive cases as determined pathologically during the operation.

CHAPTER 7

The Role of DNA Methylation as A Biomarker in Lung Cancer: Prognostic Prediction and Early Detection

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Abstract: DNA methylation is the main mechanism of epigenetic gene silencing that regulates gene expression without altering the DNA sequence, and the promoter methylation of various genes has been demonstrated to be involved in the development and/or progression of lung cancer. The growing body of evidence shows that DNA methylation of several specific genes has been identified as molecular biomarkers that accurately predict the outcome of disease. Additionally, in an effort for early detection of lung cancer, the effect of analyzing DNA methylation in several remote media has also been investigated. Here, recent achievements regarding the role of DNA methylation as a biomarker for prognostic prediction and early detection in lung cancer are reviewed.

Keywords: Biomarker, Early detection, Lung cancer, Methylation, Prognosis.

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INTRODUCTION

Despite significant advances in the detection, treatment, and understanding of molecular carcinogenesis, lung cancer is the leading cause of cancer death in many countries. Currently, the treatment strategy for the patients with lung cancer is generally decided by using TNM classification. Since the prognostic effect of chemotherapy is limited in lung cancer treatment, surgery with curative intent is the standard treatment of choice for patients at an early stage, especially stage I, non-small cell lung cancer (NSCLC); however, even after complete resection of stage I NSCLC, patients showed a wide spectrum of survival [1], indicating that it is necessary to pursue additional molecular biomarkers that predict biological characteristics and the outcome of a tumor more precisely than the TNM classification.

Since mortality from lung cancer could be reduced through early detection, various efforts for early detection of lung cancer including imaging and other types of strategies have been employed. Although a variety of investigations and screening programs for early detection of lung cancer have been conducted, each approach has lacked diagnostic specificity [2, 3]. In order to break the current deadlock, novel molecular screening strategies for early detection of lung cancer need to be investigated.

The growing body of evidence shows that aberrant methylation of CpG islands in the promoter regions is one of the major mechanisms for the silencing of tumor-associated genes through the deregulation of transcriptional profiles [4 - 6]. Recently, the identification of specific gene methylation has been recognized as a powerful molecular biomarker for cancer-treatment strategy.

ROLE OF METHYLATION AS A PROGNOSTIC BIOMARKER

The poor prognosis of patients with lung cancer is partially associated with the absence of validated molecular biomarkers that predict the outcome of the disease more accurately than the TNM system. Although many investigators have studied various clinicopathological features that might predict the outcome of curatively resected lung cancer, the clinical relevance of aberrant promoter methylation to the outcome of disease has been investigated by only a few groups. To date,

several investigations have identified that aberrant methylation of specific genes or co-hypermethylation of several genes are relevant to a high-risk of recurrence. Sandoval *et al.* [7] demonstrated that hypermethylation of *HIST1H4F, PCDHGB6, NPBWR1, ALX1,* and *HOX9A* was significantly associated with shorter recurrence of free survival in stage I NSCLC. Recent reports have identified *p16*, H-cadherin 13 (*CDH13*), *RASSF1A*, *APC*, and *FHIT* as risk factors for recurrence in patients with stage I NSCLC treated by curative surgery [1, 8]. Especially, Brock *et al.* [1] elucidated that the methylation of two or more (*p16*, *CDH13*, *RASSF1A*, and *APC*) genes in tumor tissues and mediastinal lymph nodes was associated with a lower 5-year rate of recurrence-free survivals than that for fewer than two genes.

Additionally, it is essential to develop biomarkers that can predict the efficacy of promising chemotherapy, since the identification of patients who could benefit from specific chemotherapy may be associated with a reduction of poor outcomes. Current investigations are indicating that several specific genes may influence the efficacy of anti-cancer therapy. Aberrant methylation of these specific genes would be an indicator for identifying those patients who would benefit from treatment with novel therapeutic strategies or prevent application of non-effective treatments to some patients [9].

Recently we demonstrated that methylated *BRCA1* can be a potential biomarker that predicts prognosis after curative resection of stage I NSCLC. Considering that *BRCA1* plays a role in chemotherapy-induced apoptosis, it is plausible that identification of methylated *BRCA1* could provide clinically relevant information to tailored adjuvant therapy [10].

ROLE OF METHYLATION AS A BIOMARKER FOR EARLY DETECTION OF LUNG CANCER

Since the early detection of lesions that would progress to invasive cancer could reduce the mortality from lung cancer, a variety of screening approaches for early detection of lung cancer have been investigated to improve the clinical outcome of lung cancer; however, most lack sensitivity and may involve invasive techniques. Recent reports indicated that screening using low-dose computed tomography

CHAPTER 8

Efficacy and Safety of Endoscopic Submucosal Dissection Using A Scissors-Type Knife for Early Colorectal Neoplasms

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Abstract: To reduce the risk of adverse events related to colorectal endoscopic submucosal dissection (ESD) using a conventional knife, we used a scissors-type knife (SB Knife Jr.) that allowed us to prevented unexpected muscular-layer injury.

The aims of our study were to evaluate the efficacy and safety of ESD using a SB Knife Jr. for early colorectal neoplasms. 121 lesions in 115 patients (M: F=63: 52; median age=68) were resected with ESD using a SB Knife Jr. from October 2010 to March 2014. We evaluated en bloc resection rate, complete resection rate, size of tumor, adverse events and local recurrence rate.

The sites of the neoplasms included the following: 39 lesions were located in the right colon; 38 in the left colon; and 44 in the rectum. By histological examination, 57 lesions were intramucosal cancers, 14 slightly submucosal cancers (<1,000 μm), 15 massively submucosal invasive cancers (>1,000 μm), and 35 tubular adenomas. All lesions were treated safely without unexpected incision. The en bloc resection rate was 96.7% (117/121), and the complete resection rate was 95.0% (115/121). The mean size of the resected tumors was 32.1 mm (range, 7-120 mm), respectively. With regard to adverse events, there were no perforations, and postoperative bleeding occurred in 5 cases (4.1%). All cases were controlled by endoscopic hemostasis. During a median follow-up duration of 15.5 months, local recurrence rate was 0% (0/121).

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ESD using a SB Knife Jr. can be adequately adopted as a technically efficient method for resecting early colorectal neoplasms.

Keywords: Adverse event, Early colorectal neoplasms, ESD, SB Knife Jr., Scissors-type knife.

INTRODUCTION

Endoscopic submucosal dissection (ESD) is one of the most useful methods for the treatment of early colorectal neoplasms [1, 2]. However, colorectal ESD is more technically demanding than esophageal and gastric ESD because of the anatomic features of the large intestine, which is a long luminal organ with many folds and flexures that hinder the manipulation of the endoscope for some lesions, and an intestinal wall that is thin and easily perforated [3]. Some conventional devices such as an IT knife, hook knife and needle knife are utilized for colorectal ESD. However, because these devices are used without fixing to the target, there is the potential risk of adverse events due to unexpected incision. To reduce the risk of adverse events related to ESD using a conventional knife, we used a scissors-type knife (SB Knife Jr., Sumitomo Bakelite, Tokyo, Japan) that allowed us to maintain a proper dissecting layer and prevented unexpected muscular-layer injury [4, 5]. In this study, we report on the status of ESD using a SB Knife Jr. for early colorectal neoplasms in our hospital.

PATIENTS AND METHODS

Patients

121 lesions in 115 patients (M: F=63: 52; median age=68) were resected with ESD using a SB Knife Jr. from October 2010 to March 2014 at our institute. All patients were informed about the risks and benefits of ESD and provided written informed consent for the procedure, which has been covered under health insurance since April 2012 in Japan. The indications for colorectal ESD at our hospital were based on the Criteria of Indications for Colorectal ESD proposed by the Colorectal ESD Standardization Implementation Working Group [6, 7].

We evaluated the clinicopathological features of the neoplasms, en bloc resection

rate, complete resection rate, adverse events, and local recurrence rate. Complete resection was defined as en bloc resection, where both lateral and vertical margin were negative.

ESD Procedure

Patients were sedated with intravenous nitrazepam and cardiorespiratory function was monitored. We used a single endoscope attached to a transparent tip hood with carbon dioxide insufflation. We use a PCF-Q260JI (Olympus, Tokyo, Japan) for sigmoid colon or rectal lesions, and a PCF-Q260AZI (Olympus) for lesions from descending colon to cecum. 0.4% sodium hyaluronate (Muco Up; Johnson & Johnson, New Brunswick, NJ) added to a small amount of indigo carmine was injected to the submucosal layer using a 21-gauge injection needle. A circumferential incision was made in the mucosa around the lesion with a SB Knife Jr. First, a partial dissection was performed and then further local dissection was performed after the lesion was adequately located. The tissue was also dissected along the submucoal layer with a SB Knife Jr. Basically, endoscopic hemostasis was achieved with a SB Knife Jr. However, when bleeding required repeated coagulation with a SB Knife too many times, a hemostatic forcep (Coagrasper; Olympus) was used for endoscopic hemostasis. The high-frequency generator was a VIO300D (ERBE, Tubingen, Germany), and the setting was the end-cut Q mode (effect1) for mucosal incision and submucosal dissection and a soft coagulation mode (40W) for hemostasis. We continued the procedure until the resection was accomplished.

RESULTS

Clinicopathological Features of Early Colorectal Neoplasms Resected by ESD Using SB Knife Jr.

The sites of the neoplasms included the following: 39 lesions were located in the right colon; 38 in the left colon; and 44 in the rectum. The macroscopic types of the neoplasms included the following: 23 lesions were Is/Is+IIsp/Is+IIa; 42 lesions were IIa; 5 lesions were IIa+IIc/IIc; and 51 lesions were LST-G. By histological examination, 57 lesions were intramucosal cancers, 14 slightly submucosal cancers (<1,000 μm), 15 massively submucosal invasive cancers (>1,000 μm),

CHAPTER 9

Influence of Endoscopic Stent Insertion on Detection of Circulating Tumor Cells from Obstructing Colon Cancer

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Abstract:

Objective: Self-expanding metallic stents (SEMS) have been employed as a palliative treatment for patients with obstructive colorectal cancer or as bridge to surgery for potentially resectable colorectal cancer (CRC). However, concerns have been raised about whether shear forces that act on the tumor during expansion of the stent may result in a release of cancer cells into the circulation (*i.e.*, CTCs). This study was conducted to determine whether insertion of colonic stents results in increased levels of CTCs.

Methods: Between October 2013 and November 2013, three patients who underwent colonic stent insertion for obstructing colorectal cancer were studied. To detect viable CTCs of CRC, we employed a TelomeScanF35 detection system, which was constructed with a GFP-expressing attenuated adenovirus, in which the telomerase promoter regulates viral replication. 7.5ml of peripheral blood samples were obtained before and after stent insertion and after operation.

Results: Three patients were inserted SEMS and performed laparoscopic surgery. In

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one of these 3 patients, CTCs increased after stent insertion and continued in expression after the operation.

Conclusions: This study demonstrated that endoscopic stent insertion results in dissemination of tumor cells into the peripheral circulation. Although oncological consequences were not examined in this small population, viable CTC detected by TelomeScanF35 may represent a suitable prognostic marker to stratify the risk of patients with distant metastasis following stent insertion for obstructing colon cancer.

Keywords: Bridge to surgery, Circulating tumor cells, Obstructing colon cancer, Self-expanding metallic stent, TelomeScan F35.

INTRODUCTION

Colorectal cancer (CRC) is the second most common cause of cancer mortality in developed countries and has a high mortality rate [1]. In Japan, CRC is the third leading cause of cancer death [2]. When obstructing colorectal cancer is detected, emergency surgery was once the standard choice. Self-expanding metallic stents (SEMS) have been employed as a palliative treatment for patients with obstructive colorectal cancer or as bridge to surgery for potentially resectable CRC. Recently, SEMS has become the treatment of choice in many centers with facilities available to release colonic obstruction with primary anastomosis and no stoma. Further, SEMS reduces the dependence on surgeons for emergency surgery [3 - 5]. There are concerns, however, about whether shear forces that act on the tumor during expansion of the stent may result in a release of cancer cells into the circulation (*i.e.*, circulating tumor cells: CTCs). Small populations of cancer cells in the circulatory system that have detached from the primary tumor are designated as CTCs. Many groups have reported that CTC detection was associated with CRC progression and poor prognosis. The present study was conducted to determine whether insertion of colonic stents results in increased CTC levels.

MATERIALS AND METHODS

Virus

TelomeScanF35 is a telomerase-specific, replication competent adenovirus

developed by Sakurai *et al.* (Osaka Univ., unpublished data). The *hTERT* promoter region is inserted into E1A and E1B genes linked with internal ribosomal entry site and *gfp* genes under the CMV promoter are inserted into the E3 region. To ensure infection of virus into broad types of cells, the adenovirus type 5 fiber region of the virus is converted to type 35. The miR-142-3p target sequence is inserted into the 3' region of E1B and E3 genes to suppress replication of the virus and expression of *gfp* genes within blood cells. Virus concentration is determined by measuring the virus solution by absorption spectrometer (GE healthcare) at 260nm absorbance. Virus GFP expression was confirmed by observing cells infected with the virus under fluorescence microscopy (IX71, Olympus). The virus was stored at -80°C until use.

Reagents and Antibodies

Mouse monoclonal anti-human CD45 antibody (clone HI30, Biolegend), mouse monoclonal anti-human CK antibodies (clone A53-B/A2 and C-11, Biolegend), and rabbit polyclonal anti-vimentin antibody (abcam) were used as primary antibodies. Alexa Fluor 555 goat anti-mouse IgG (Invitrogen), and Alexa Fluor 647 goat anti-mouse IgG (Invitrogen) were used for CD45 staining. Alexa Fluor 350 goat anti-rabbit IgG (Invitrogen) for vimentin staining was used as secondary antibodies.

Blood Sample Processing

Preparation of samples for CTC detection using TelomeScanF35 was modified from the methods originally established by Kojima *et al.* [6] as shown in Fig. (**1**). Briefly, peripheral blood was recovered into collection tube containing a CPD (citrate-phosphate-dextrose) buffer and stored at room temperature until shipment. The processing of blood samples was started within 24 hours from blood collection. To remove erythrocytes, platelets, and serum from whole blood, blood was mixed with red blood cell lysis buffer containing ammonium chloride for 5-10 minutes at room temperature and then centrifuged at 300 x *g* for 5 minutes. After centrifugation, supernatant was removed by decantation. A cell pellet was gently washed twice with DMEM supplemented with 10% FCS and penicillin/streptomycin. Cells were mixed with 1E9 VP of TelomeScanF35 and

CHAPTER 10

Clinical Outcome of Liver Resection in Single Center Experience: Laparoscopic *versus* Open Procedure

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Abstract: Minimally invasive surgery for liver resection still remains controversial. The present study was designed to compare open *versus* laparoscopic liver resection (LLR).

Between January 2012 and December 2013, LLR was performed on 30 patients at our institution. We performed partial hepatectomy through a pure laparoscopic method and anatomical hepatectomy by a laparoscopy-assisted method. We performed a retrospective review of these patients.

The study comprised 25 men and 5 women with a mean age of 70.5 years. All patients had a liver function of Child-Turcott-Pugh classification A. The mean diameter of the tumors was 20.8 mm. Thirteen cases were resected by the pure method and 17 cases underwent the hybrid method. Nineteen patients underwent partial hepatectomy, 8 patients underwent subsegmentectomy, and 3 patients underwent segmentectomy. Mean intra-operative bleeding was 403.7 ml, and mean operating time was 316.4 min. No patients developed severe postoperative complications. We compared 19 laparoscopic partial hepatectomies (LLR-P) with 9 open partial hepatectomies. Although, no significant differences were observed in perioperative factors, both length of hospital stay and the frequency of analgesic administration were significantly

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reduced in LLR-P. Furthermore, we compared 13 laparoscopic (sub) segmentectomies (LLR-S) with 11 open cases. The operation time of LLR-S were longer than those of open cases, whereas no significant differences were detected in blood loss in these procedures. All length of hospital stay favored the LLR-S group.

In these series comparing laparoscopic and open liver resections, there were no severe complications observed in the LLR group and there was significant rapid recovery in the LLR group, even for resections involving (sub) segmentectomy by the hybrid method.

Keywords: Hepatectomy, Laparoscopic liver resection, Laparoscopyassisted liver resection, Minimally invasive surgery.

INTRODUCTION

Laparoscopic surgery has become widespread in the field of abdominal surgery over the last two decades. Though laparoscopic liver resection (LLR) was first reported in 1992 [1], extensive introduction of LLR was delayed because there were many technical difficulties. Recently, LLR has become more popular along with the advancement of endoscopic devices. However, the advantage of minimally invasive surgery for liver resection still remains controversial. We introduced LLR in 2012 as minimally invasive surgery for hepatic neoplasm. In this study, we summarized our short-term outcomes for LLR and compared LLR with open liver resection during the same research period.

METHODS

Between January 2012 and December 2013, 30 patients underwent LLR at the Department of Surgery, Kure Medical Center and Chugoku Cancer Center. Before surgery, we informed patients about the risks and benefits of LLR and received informed consent. In our strategy, we performed partial hepatectomy thru a pure laparoscopic method (pure-LLR) and anatomical hepatectomy by a laparoscopy-assisted method (hybrid-LLR), except for cases of lateral segmentectomy. All procedures were performed in a lithotomy position regardless of the tumor location. We placed 4 or 5 ports (usually use two 12 mm, one or two 5 mm ports with a 12 mm port for a camera on the umbilicus). We applied Pringle's maneuver for all cases. In the hybrid method, after mobilization of the right or left lobe of

the liver and preparation with cotton tape around the hepatoduodenal ligament for Pringle's maneuver, a laparotomy was placed on the right subcostal if a tumor was located in the right lobe of the liver and on the center of upper abdomen if the tumor was located in the left lobe (Fig. **1**).

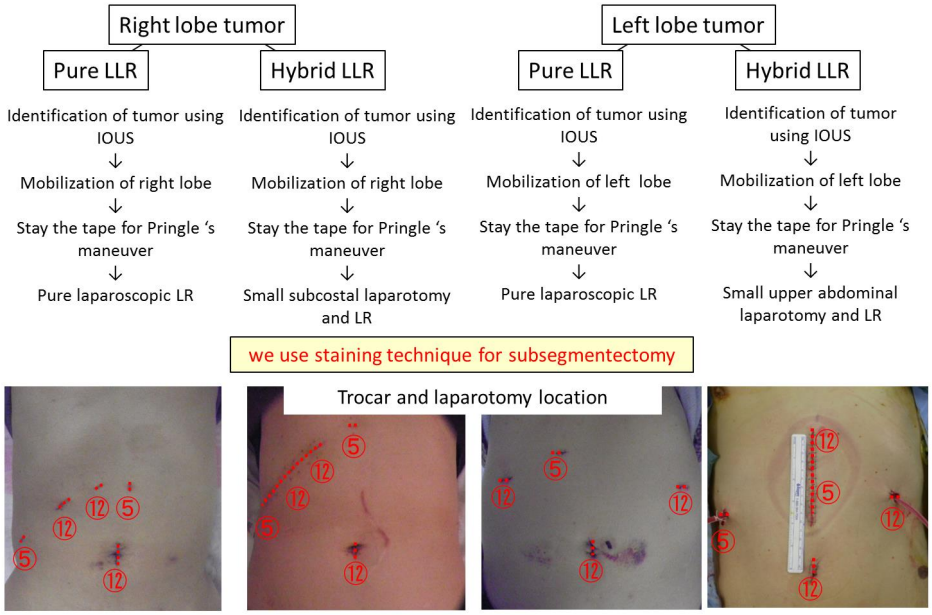

Fig. (1). Our strategy and brief procedure for laparoscopic liver resection.
IOUS, intraoperative ultrasonography; LLR, laparoscopic liver resection.

We performed a retrospective review of these patients and compared 19 laparoscopic partial hepatectomies (LLR-P) with 9 open partial hepatectomies in the same research period. Furthermore, we compared 13 laparoscopic (sub) segmentectomies (LLR-S) with 11 open (sub) segmentectomies. Statistical analysis was done using Statmate V (ATMS, Tokyo, Japan). Student's test was used for comparisons of two continuous variables and Chi-square test was used for categorical data. A P value of <0.05 represented a statistically significant result.

RESULTS

The study comprised 25 men and 5 women with a mean age of 70.5 years. Twenty-seven patients had hepatocellular carcinoma (HCC), two had metastatic

CHAPTER 11

Transcatheter Arterial Chemoembolization for Hepatocellular Carcinoma: Does TACE Have a Future?

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Abstract: Transcatheter arterial chemoembolization (TACE) is the interventional technique and mainstay of treatment for unresectable hepatocellular carcinoma (HCC). TACE is indicated mainly for unresectable HCC, cholangiocarcinoma, and hepatic metastases. In this method if used worldwide; however, many regimens, embolic materials, and techniques exist. The clinical results are also controversial. We present the history and the status of TACE for HCC.

Keywords: HCC, History, Interventional radiology, Lipiodol, TACE.

HISTORY

Doyon *et al.* [1] reported the first case of transcatheter arterial embolization (TAE) for hepatocellular carcinoma (HCC) in 1974 from France; this report was followed by Goldstein *et al.* [2] in 1976 from the United States. In Japan, Yamada *et al.* [3] established this method by performing TAE for 120 HCC cases between 1977 and 1982. In the early times, iodized oil (lipiodol or ethiodol) was not used;

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however, several reports [4 - 6] in 1985 demonstrated that a mixture of lipiodol and anticancer drug was effective. Lipiodol turned out to play a role, not only as an anticancer drug carrier, but also as an embolic agent. Moreover, it featured a visual marker on CT after TACE.

Initially, transcatheter arterial chemoembolization (TACE) was called HAE (hepatic artery embolization), THAE (transcatheter hepatic arterial embolization) [7], TAE or Lp-TAE (Lipiodol-TAE), *etc*. After advent of the microcatheter, segmental or subsegmental Lp-TACE was also developed. Additionally, after 2000, the name TACE gradually became a global standard, although this method has not yet been precisely defined. In the 1980s, a large part of the papers and presentations on TACE for HCC came out of eastern Asia. However, TACE has become a more common procedure because of the increase in HCC patients with hepatitis C in the West. Today, many papers are coming from all over the country. While these are the main factors of standardization, I presume that one reason the term TACE (phonetic sign: teis) is used because native English speakers can pronounce it easily and it sounds comfortable.

INDICATION

We have conducted a dozen liver staging systems worldwide. Georgiades CS *et al.* [8] recommended the Child-Pugh nominal liver staging system as the most accurate in predicting survival of patients with unresectable HCC treated with TACE.

The Barcelona Clinic Liver Cancer guideline (or the Japanese guideline) [9, 10] shows that TACE is regarded as a palliative treatment for patients who cannot undergo surgical resection, transplantation, or radiofrequency ablation (RFA) therapy. Patient with performance statuses of 0, Child-Pugh A or B, and multi-nodular hypervascular HCCs are the best candidates for TACE. However, even when patients are suitable for curative treatment, we sometimes have to perform TACE because of age. We are becoming an elderly society, especially in Japan. Indeed, a quarter of the patients in our hospital that undergo this procedure are over 80 years old. Therefore, other countries will also have more elder patients who are eligible for TACE.

TACE for advanced HCC with invasion of the main portal vein (PV) is relatively contraindicated because of risk of liver failure. However, Chern *et al.* [11] reported that TACE for HCC with major PV invasion was safe and effective.

Recently, Kim *et al.* [12] also showed that TACE for advanced HCC with invasion of the hepatic vein and inferior vena cava were safe and effective. Fatal pulmonary tumor embolism rarely occurs after TACE [13]. Therefore, well-rounded informed consent is required.

Borderline liver function, poor performance status, extrahepatic metastases, *etc.*, are also relative contraindications for TACE [14]. Therefore, a careful and gentle procedure is needed.

PROCEDURE

Right femoral artery (rarely brachial or radial artery) is selected as the puncture site. Under local anesthesia, a 3-4 French size catheter is engaged into the celiac artery with the Seldinger technique. Celiac angiogram shows the location of HCC and the route to HCC. A 1.9-2.8 French size microcatheter is coaxially advanced into the feeding artery. After selective angiogram, a mixture of an anticancer drug (ex.,cisplatin or epirubicin) and lipiodol is administered. Finally, the feeding artery is embolized with 1-2 mm gelatin sponge particles.

HCCs are sometimes supplied by extrahepatic arteries mainly because of repeated TACE. In particular, the inferior phrenic artery easily becomes the main parasitic artery. Therefore, TACE is required for these feeding arteries.

After catheter withdrawal, 10 minutes of compression is sufficient for hemostasis with a 3F catheter. If you would like to use a closure device, you can (This device is not covered by medical insurance in Japan, so we do not use it).

REGIMENS

We have two major protocols when using anticancer drugs among many regimens for TACE (unfortunately, there is no high-level evidence as to whether anticancer drugs are effective). One protocol is a single-drug use and the other is a triple-drug use (so-called triple cocktail) [14, 15]. The former includes the anthracycline

CHAPTER 12

Immunotherapy for Pancreatic Cancer: Clinical Relevance of α-gal Epitope/Natural Anti-gal Antibody Reaction

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Abstract: Pancreatic cancer remains lethal and most are resistant to traditional therapies. New therapeutic approaches, such as immunotherapy are in urgent demand. Anti-Gal antibody (Ab) is known as the most abundant natural Ab in humans, and the anti-Gal ligand called "α-gal epitopes" with the structure Galα1-3Galβ1-4GlcNAc-R is expressed as a major carbohydrate antigen. Pancreatic cancer cells or their lysate may express α-gal epitopes. Subsequent vaccination with such processed cell membranes result in *in vivo* opsonization by anti-Gal IgG in cancer patients. The interaction of the Fc portion of the vaccine-bound anti-Gal Ab with Fc cγ receptors of APC induces an effective uptake of the vaccinating tumor cell membranes by the APC, followed by the effective transport of the vaccinating cancer membranes to the regional lymph nodes, and processing and presentation of the cancer-associated antigens. Activation of cancer-specific lymphocytes elicits an immune response to eradicate the residual cancer cells that remain after completion of standard therapy in some patients. This review

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focuses on this unique antigen/antibody system (α-gal epitope/natural anti-Gal Ab) and shows advances in immunotherapy for pancreatic cancer.

Keywords: 3 Galactosyltransferase, α-1, α-gal epitopes, Immunotherapy, Pancreatic cancer, Universal vaccine.

INTRODUCTION

The incidence of pancreatic cancer in the United States in 2012 was estimated at 43,920 new cases and 37,390 deaths [1]. This cancer is common worldwide. The extremely poor prognosis comes about due to late diagnosis, resistance to conventional chemotherapies, and high immunosuppression [2]. The FOLFIRINOX regimen is a combination of fluorouracil (5-FU), leucovorin, oxaliplatin and irinotecan. This regimen and gemcitabine are the only currently known treatments effective against pancreatic cancer [3, 4]. However, patient survival treated with these regimens remains marginal. Although an immunotherapy against tumor-associated antigens (TAAs) is promising, it is limited by the activity of cytotoxic T lymphocytes (CTLs) in pancreatic cancers. Therefore, other modalities of immunotherapy are needed for the treatment of pancreatic cancer [5, 6].

In order to develop an effective immunotherapy against pancreatic cancer, it is essential to identify an appropriate tumor antigen.

Natural anti-Gal antibody (Ab) is the most abundant Ab in both normal humans and patients with malignancies. It constitutes ~ 1% of immunoglobulines and is found as IgG, IgM and IgA isotypes. About 1% of B cells have been reported to produce anti-Gal Ab in human [7 - 11]. These B cells (anti-Gal B cells) are mostly in a quiescent state within the lymph nodes and spleen, but they produce natural anti-Gal Ab when present along the gastrointestinal tract because bacteria in the natural flora continuously stimulate them [12]. Several heavy-chain genes primarily of the VH3 immunoglobulin gene family encode anti-Gal in humans [13, 14]. Anti-Gal Ab binds to α-gal epitopes (Galα1-3Galβ1-4GlcNAc-R) on cell surface glycolipids and glycoproteins, and then the Fc portion of Ab binds to FcγR III on dendritic cells and macrophages. These strategies explain the effective phagocytosis of anti-Gal-Ab-opsonized cells by antigen presenting cells (APC) [9,

15 - 18]. In this review, we show our recent data for vaccination and interaction between α-gal epitopes and anti-Gal Ab in addition to addressing the relevant immunotherapies and their basic problems in pancreatic cancers.

DISTRIBUTION OF A1, 3GALACTOSYLTRANSFERASE (A1,3GT), A-GAL EPITOPES AND ANTI-GAL ANTIBODY IN MAMMALS

The α-gal epitope has a unique carbohydrate structure. It is absent in humans, but is naturally expressed on glycolipids and glycoproteins in non-primate mammals, prosimians, and New World monkeys [19]. All mammals synthesizing α-gal epitopes are immunotolerant to it and cannot produce anti-Gal Ab [20 - 25].

High titer of anti-Gal Ab is found in sera of non-immunocompromised humans. Anti-Gal Ab is estimated as a polyclonal Ab since many related genes encode the anti-Gal heavy chain [26]. However, anti-Gal Ab can specifically interact with α-gal epitopes on glycolipids and glycoproteins. Anti-Gal Ab is ubiquitous in humans and anti-Gal activity is found in cancer patients with solid tumors such as pancreatic cancer and in patients with B cell lymphoma [27].

TARGETING WHOLE-CELL VACCINES TO ANTIGEN PRESENTING CELLS

Inoculation of patients with irradiated cancer cells has been applied to pancreatic cancer to develop cancer cell vaccines. The advantages of this method have been described previously [28 - 30], and can be briefly summarized as follows: (i) specific cancer antigens are unnecessary, (ii) immune responses to multiple cancer antigens [31], (iii) no limitation due to patient HLA background [30, 32], and (iv) easy modification of the cancer cell vaccine platform. Pancreatic cancer cell vaccines induce a $CD8^+$ T cell response with no relation to the HLA match between the tumor vaccine and recipient with the occurrence of cross priming [29, 20]. Mesothelin is a cell-surface glycoprotein and it is a particular cancer vaccine target. Mesothelin is expressed at a low level in healthy pancreatic tissue, but at a high level in pancreatic cancer tissue [33]. Uptake of cancer cells or cancer cell membranes by professional APCs, processing of TAA molecules, presentation of TAAs antigenic peptide on APCs in association with MHC class II molecules for activation of specific $CD4^+$ helper T cells, and in association with MHC class I

CHAPTER 13

Evaluation of Cervical Liquid-Based Cytology in Glandular Abnormalities of Japanese Women

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Abstract: Liquid-based cytology (LBC) has been adopted widely throughout the world. However, diagnosis for glandular abnormalities still remains difficult.

LBC and conventional Papanicolaou smear (CS) were compared for cytological diagnosis by a split- sample method. The results showed that LBC reliability is near to that of CS for screening of glandular malignant lesions.

Herein, we detail the results of 69 cases analyses that were diagnosed as a glandular abnormality by the TP method among a total of 11,092 cases. The TP method resulted in the same diagnosis of CS as either AGC or adenocarcinoma in 54 cases (78.3%), up-grading in four cases (5.8%), and down-grading in seven cases (10.1%). From detailed analysis of individual cases, it is thought that one cause of discrepancy was the characteristic morphology of the cells and clusters that appeared in TP samples.

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These results suggest that adequate knowledge of the characteristic features of glandular lesions in LBC could contribute to a more accurate diagnosis and to shifting to LBC for cervical cancer screening.

Keywords: Atypical glandular cell, Conventional Pap smear, Liquid-based cytology, The Bethesda System, Uterine cervix.

INTRODUCTION

Despite a decrease in uterine cervical cancer in developed countries, it is still a fatal cancer among women all over the world [1 - 6]. In recent years, increasing numbers of facilities in Japan have begun using liquid-based cytology (LBC) in combination with The Bethesda System (TBS) because of the equivalency in diagnostic accuracy and improved quality of samples compared to conventional Papanicolaou smear (CS) tests [7 - 16].

However, one obstacle to LBC popularization comes from the difficulty in arriving at a diagnosis of glandular abnormality without distinct criteria in LBC. Recently, malignant or precancerous diseases of uterine cervical glands have increased; the reported incidence of adenocarcinoma and adenosquamous carcinoma in Japan was 21.9% in 2010. Higher mortality rates have been reported in many studies of cervical adenocarcinoma [17 - 22].

We recently performed a multi-institutional study of TBS application using both CS and LBC, and simultaneously investigated HPV infection to investigate diagnostic accuracy for glandular abnormality in an interim report [23, 24]. In the present study, we concluded that LBC is equally credible compared to CS in terms of glandular abnormalities screening.

Atypical glandular cells (AGC) were newly adopted into TBS in 2001 instead of glandular dysplasia because the biologic significance of glandular dysplasia is still unknown in terms of being precancerous lesions of adenocarcinoma [25, 26].

Diseases cytologically diagnosed as AGC possibly include reactive atypia, repair changes, radiation atypia, tubal metaplasia, cytological changes in pregnancy, and adenocarcinoma *in situ* (AIS) with marginal atypia, which makes diagnosis

difficult.

Here, we present the results of a comparison study by a split-sample method between LBC with ThinPrep® devices (Hologic, Marlboruogh, Mass; TP) and CS for cytological diagnosis with a total of 11,092 cases in the final report of the study. The correlation between TP and CS in the cytological diagnosis of glandular legions was determined to investigate problems in the examination of individual cases.

MATERIALS AND METHODS

In this study, 11,092 cases were retrieved from eight institutions including the National Hospital Organization, Kure Medical Center and Chugoku Cancer Center (NHOKMCCCC) from November 2007 to July 2011. Case collection and the analysis method were described in a previous report [24], in which analysis results from 4,522 cases enrolled through July 2009 were reported. In brief, samples were obtained using an EndoCervex-Brush® (Rovers Medical Devices B. V., Oss, The Netherlands) and CS slides were made at each institution. Then, residual cells in TP preservative were sent to NHOKMCCCC, followed by production of TP slides with Papanicolaou staining. Human papillomavirus (HPV) DNA testing was performed using the multiplex PCR method [27] with residual cells in the TP preservative at GeneticLab Co., Ltd (Sapporo, Japan).

The specimens were screened by cytotechnologists and TBS was done as a blinded experiment. All screeners engaged in this study acquired certification for the ThinPrep test, presided over by Cytyc Co. (Marlborough, MA) prior to the study.

RESULTS

Table **1** shows a comparison between TP and CS methods for 11,092 cases. According to TBS, the cytological diagnosis was made by a TP or CS method in 11,081 or 11,046 cases, respectively, after excluding unsatisfactory samples. Satisfactory cases for both methods were 11,039 and average patient age was 46.1 years old. The number of positive cases were 1,190 (10.8%) by TP and 1,137 (10.3%) by CS. Positive cases by TP were classified as follows: 173 (1.6%) cases

CHAPTER 14

Clinical Application on Telomere Biology for Cancer

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Abstract: Telomere dynamics have been reported extensively as a critical component of cancer, and established evidence exists an important relationship between telomere biology and cancer. Although more than 85% of all carcinomas rely on telomerase activity to maintain stable telomere length, most normal somatic cells are telomerase negative. Therefore, effective methods for specific diagnostic and prognostic utility by telomerase detection have been described, and, to date, ideal targeting telomerase therapy with minimal adverse effects on normal somatic cells have been attempted. In this mini review, we summarize the role of telomeres and telomerase in cancer, clinical use, and targeted telomerase cancer therapies.

Keywords: Adverse effects, Cancer, Sarcoma, Telomerase, Telomere.

TELOMERE AND TELOMERASE

Human telomeres form the ends of chromosomes composed of specialized structures containing $(TTAGGG)_n$ repeats [1, 2] and an associated protein complex termed shelterin [3]. The shelterin complex is thought to be important in the protection of chromosome ends from end-to-end fusions and degradation of forming t-loop like structures [4]. Lagging strand DNA synthesis at the end of chromosomes cannot be completed. This situation, called an 'end-replication

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problem', results in the progressive shortening of telomeric repeats with each cell division [5]. Telomerase contains an RNA-dependent DNA polymerase, which is a cellular reverse transcriptase that contributes to the maintenance of the telomere by adding new DNA repeats onto telomeric ends [6, 7], which enables cellular immortality [8, 9].Telomerase holoenzyme core components are a human telomerase reverse transcriptase (hTERT), which is the catalytic reverse transcriptase [10] and an associated template RNA (hTR or hTERC) [10, 11].The close relationship between hTERT mRNA expression and telomerase activity suggests that quantification of the mRNA expression of hTERT could be used as an alternative measure of telomerase activity [11 - 13]. Telomerase must be limiting to maintain normal telomeres; however, overexpression of both hTERT and hTR causes continuous elongation of telomeres in cancer cells [14].

TELOMERES MAINTENANCE MECHANISMS IN CANCER

Two types of telomere maintenance mechanisms have been described in human tumors: telomerase activation and alternative lengthening of telomeres (ALT) [15, 16]. Telomerase is expressed in germline cells, but not in most somatic cells [17]. The expression of telomerase activity has been reported in more than 85% of carcinomas [18, 19], and telomere maintenance by telomerase is regarded an important mechanism in evading senescence [8, 9]. Although carcinoma cells basically engage a mechanism to maintain stable telomere length by reactivating or up-regulating telomerase activity, many types of sarcomas have been reported to have ALT- telomere recombination pathways that usually exhibit a remarkably elongated and heterogeneous telomere length in the absence of telomerase activation [20 - 22]. The co-existence of ALT and telomerase activity is unlikely in the vitro study [23], however, evidence of the presence of both ALT and telomerase activity has been shown especially in sarcoma [24 - 27]. The ability of cancer cells to replicate through telomerase activation has been referred to as a hallmark of cancer [28], therefore, the telomere maintenance mechanism could be necessary for tumor progression.

CLINICAL UTILITIES OF TELOMERASE AND TELOMERE

The detection of telomerase activity and hTERT has been evaluated on fresh

frozen tumor biopsies, fluids, secretions, aspirates, and peripheral blood using available assays [29, 30]. Abundant experimental data also exists on telomerase activity and hTERT expression in various clinical samples as a potentially sensitive biomarker for various cancers and the early detection of cancer cells, specifically, these marker can distinguish malignancies from benign tumors and serve as a prognostic indicator [19, 31 - 55]. In terms of telomere length distribution, a number of assays exist to measure telomere length [56 - 60]. Although telomere reduction is implicated in the incidence of many cancers, elongated telomeres consistent with ALT are evident in several types of sarcomas. Researchers have also reported the telomere maintenance mechanism as being a prognostic factor for patients with sarcomas, and ALT is positivity correlated with worse survival of patients with several types of sarcomas [22, 24 - 27, 61 - 69]. In the near future, detection of telomerase, or hTERT, and evaluation of telomere distribution are likely to become common methods for diagnosis, prediction of prognosis, and grading of malignancies. The identification of circulating tumor cells (CTCs) in the blood is a clinically useful biomarker for early detection of cancer, prognosis, disease progression, and surrogate marker of treatment effects in various malignancies [70 - 73]. We recently conducted a study to detect CTCs in peripheral blood with sarcoma and several carcinoma patients using a green fluorescent protein-expressing attenuated adenovirus vector in which the hTERT promoter regulates viral replication (OBP-401; TelomeScan [74, 75]). This assay is a remarkably simple using an *ex vivo* method that was able to detect viable human CTCs in the peripheral blood, and may open a new frontier of clinical utility (early diagnosis, prognostic marker) for cancers.

TELOMERASE-TARGETED THERAPY

Targeting telomerase is considered a very attractive approach to cancer therapeutics. Most carcinomas not only express telomerase, but also have very short telomeres, whereas telomerase activity is undetectable in most normal somatic cells [17, 76]. Differences in telomerase expression and telomere length between normal and tumor tissues suggest that targeting telomerase would have minimal side effects and be relatively safe. To date, numerous approaches to targeting telomerase and telomeres have been described [77 - 79]. Clinical trials to target telomerase biology are ongoing with telomerase inhibitors [80], gene

CHAPTER 15

Cancer Counseling and Pathology Clinic

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Abstract: In Japan, the Cancer Control Act was passed in 2006. Consequently, our center has been proactively conducting palliative care promotions. Concerning health insurance coverage, the "Cancer Counseling Fee" became payable for health insurance remuneration as of April 2010. The cancer counseling fee covers the service provided to patients diagnosed with malignant tumors in an environment with sufficient consideration of their psychological states. Physicians with experience in cancer treatment and full-time nurses with experience with cancer patients collaborate to explain and consult with patients to allow them to select a line of treatment upon full understanding of and consent to diagnoses and treatment methods. At our center, cancer patients are offered cancer counseling in all clinical departments, and an explanation by a pathologist in a pathology clinic plays an important role in performing well-organized cancer counseling.

When explaining the diagnosis to patients in the pathology clinic, the pathologist also attends to the patients' emotions while listening to their anxieties and empathizing with their pain. Patients that accurately understand the conditions of their illness and the treatment's policies and effects are more likely to consent to the explanation by the attending physician and proactively accept the standard therapy by the attending physician, which increases medical compliance. The combination of the explanation by

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a pathologist in the pathology clinic and cancer counseling by a nurse can improve the mental disposition of these patients.

Keywords: Cancer counseling, Diagnosis, Informed consent, Malignancy, Pathology clinic.

INTRODUCTION

The primary author began hospital ward duty as a certified palliative care nurse in 2005, and as of April 2010, she has been offering cancer counseling at the National Hospital Organization (NHO), Kure Medical Center and Chugoku Cancer Center (KMCCCC) as a full-time palliative care nurse. She has conducted cancer counseling together for three years with Dr. Taniyama, K who introduced the pathology clinic into the KMCCCC. In this article, we will present through cases that combination of courteous and easily understanding explanations of pathological diagnosis by a pathologist and psychological support by a counseling nurse is effective in supporting patient's mental condition to be better and good decision-making that affects the cancer patient's treatment.

CANCER COUNSELING

In Japan, the Cancer Control Act was passed in 2006. This act suggests that "the promotion of palliative care after cancer diagnosis" is the first issue that must be addressed. KMCCCC actively promotes palliative care and holds a "Workshop for Physicians Engaged in Cancer Treatment" annually. Regarding health insurance coverage, the "Cancer Counseling Fee" became payable for health insurance remuneration as of April 2010. Counseling for patients was originally a practice conducted by general physicians and nurses, but the new legislation states that health insurance remuneration will cover only when this service is provided to the patients diagnosed with malignant tumors in an environment with sufficient consideration of the patients' psychological states, with physicians that have experience in cancer treatment and full-time nurses that have experience in nursing cancer patients collaborating to explain and consult with patients to allow them to select a line of treatment upon full understanding of and consent to diagnoses and treatment methods.

Physicians that have experience with cancer treatment are those that have completed the palliative care workshop for physicians treating cancer patients. As of 2013, KMCCCC has 68 physicians that qualify. In addition, nurses that have experience caring for cancer patients are cancer-specialist or certified nurses that have completed the Japanese Nursing Association's Certified Nursing Curriculum or the Japanese Nursing Association's Certified Specialist Nursing Curriculum. KMCCCC employs 15 such nurses including the primary author.

Counseling, according to a general dictionary, is defined as "discussing an individual's psychological problems, such as worry and anxiety, and providing assistance and advice for solving them". Additionally, medical definition of counseling states that counseling is "a process of support, including the elucidation of psychological problems and cooperative consideration of solutions to these problems," and "the minimum requirement is to not persuade the client, but to listen to and empathically understand the client". Medically, cancer patients experience the following psychological processes:

1. Immediately after experiencing major stress, people become devastated. The mental disturbance continues after the stress. Most people experience this change.
2. Ten to 14 days later, people become able to adapt to the situation well. This state is called the adaptive state [1].

After being informed of a major illness, the mind passes through the stages of shock, instability, and adaption along with the passage of time. However, in some cases people fail to adapt, which leads to an adjustment disorder. To avoid the state of an adjustment disorder, clients that are thrown into an unexpected situation require support that alleviates the shock. Organizing information is important during the period of unstable chaos. It is important for the patient to take the time they need to take and to progress to the period of adjustment that will lead them to their own answers.

The pain in mind relates to the largeness of the gap between the objective situation and subjective thoughts, desires, and values, and larger gaps are associated with greater pain [2]. This gap is equal to the size of the shock towards

CHAPTER 16

Late Endocrine Effect for Childhood Cancer Survivors

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Abstract: In recent years, the treatment and diagnosis of childhood cancer has led to dramatic improvements in survival rates. Approximately, 75-80% of affected children survived a malignant disease. However, the improvement in prognosis has been achieved at the expense of serious late effects from cancer and cancer treatment. Endocrinological late effects are the most common problem and were found in approximately 20-30% of child cancer survivors (CCSs). Endocrinologists should participate in the follow-up of CCSs in collaboration with pediatric oncologists. Further, long-term follow up is necessary for CCSs because treatment-related complications may occur during childhood and many years after therapy.

Keywords: Child cancer survivors, Growth impairment, Late effect, Malignant disease, Treatment.

INTRODUCTION

In recent years, the treatment and diagnosis of childhood cancer has led to dramatic improvement in survival rates. Approximately, 75-80% of affected children survive a malignant disease [1, 2]. The number of childhood cancer survivors (CCSs) has been increasing in Japan as well as other countries, including the USA and Europe [3]. The improvement in prognosis, however, has been achieved at the expense of serious late effects from cancer and cancer

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treatment done with radiotherapy, surgery, and/or chemotherapy. The all cases mortality of CCSs is significantly higher, compared to an age-adjusted background population, and overall cumulative mortality increases over time. Although the risk of death due to primary malignancy decreases with an increase in time from cancer treatment, the mortality rate occurring 20 years after diagnosis has elevated. Other causes for late mortality are becoming important with time [2].

Follow-up should ensure that children surviving cancer achieve normal or maximal growth, maturation and psychosocial adaptation. Adverse outcome has shown organ failure (*e.g.*, cardiac, pulmonary, gonadal, pituitary gland, thyroid, adrenal gland), short stature, decreased fertility, impaired cognitive function, second neoplasm, difficulties obtaining employment and insurance, and overall reduction in quality of life.

The survival rate of CCSs has increased over the last two decades. However, 50-75% of CCSs were found to be at least one chronic medical problem. Endocrinological late effects are the most common problem and were found in approximately 20-30% of CCSs [4, 5]. The frequency of endocrine disorders was significantly higher in patients with brain tumors [6].

Endocrine disorders include growth hormone deficiency (GHD), obesity, osteopenia, thyroid dysfunction, adrenocortical dysfunction, parathyroid gland, central diabetes insipidus and gonadal function disorders (hypogonadism, precocious puberty).

HYPOTHALAMIC-PITUITARY HORMONE DEFICIENCY

CCSs patients who have received cranial irradiation, brain tumors, acute lymphoblastic leukemia (ALL), nasopharyngeal carcinoma or total body irradiation (TBI) in preparation for bone marrow transplantation (BMT) are at risk of developing deficiency of one or more pituitary hormones. GH secretion is the most sensitive to radiation damage, being the first of the six anterior pituitary hormones. Almost all patients who were irradiated for pituitary tumors had developed growth hormone deficiency at five years after irradiation.

The next most sensitive hypothalamic-pituitary hormones are gonadotropins and adrenocorticotropic hormone (ACTH). There is a clear dose relationship between cranial irradiation and development of hypopituitarism. And, hypothalamus appears to be more sensitive than the pituitary gland. Along with a greater radiation dose, there is observation of gradual deficiency of all pituitary hormones (GH, gonadotropins, ACTH, thyroid stimulate hormone, antidiuretic hormone) [7].

GROWTH IMPAIRMENT

Cancer therapy can damage normal tissue and affect the function of vital organs. The most common symptom is growth abnormality following cancer therapy. Radiotherapy can have late effects on growth due to damage to growing bone and tissue. The most common cause of growth impairment is GHD.

GHD may occur as a direct consequence of cranial irradiation, although other possible adverse factors affecting growth may be due to direct radiation damage to the spine, or the long bone, malnutrition, cytotoxic drugs, therapy with corticosteroids or precocious puberty.

GROWTH HORMONE DEFICIENCY

GH has the highest sensitivity to radiation damage of all pituitary hormones, being the first of the six anterior pituitary hormones to be lost after cranial irradiation. There is a clear dose relationship between cranial irradiation and the development of hypopituitarism. The hypothalamus is more radiosensitive than the pituitary gland, and most induced growth hormone deficiencies seem to result from disruption of the GHRH/somatrostatin feedback mechanism. Until growth is complete, the Children Oncology Group (COG) recommends that children treated with cranial irradiation should undergo screening to monitor height, Body Mass Index (BMI) percentile, bone mineral density (BMD), sexual maturity rating, psychological status and quality of life (QOL).

GHD can be treated with GH replacement therapy. GH replacement therapy can improve the chances of a GHD child making up a height deficit and achieving a better final height [8]. The clinical features of GHD include increased fat mass

CHAPTER 17

Changes in Esophageal Cancer Treatment Over the Past Decade at Our Hospital

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Abstract: An esophagectomy with three-field lymph node dissection has been performed widely in Japan, but multidisciplinary therapy that uses a combination of radiation therapy and chemotherapy is necessary to improve the prognosis in patients with advanced esophageal cancer. Several new techniques have contributed to reducing post-operative complications and we have introduced some techniques into the treatment of esophageal cancer at our hospital.

Keywords: Neoadjuvant chemotherapy, Oral care, Prone position, Respiratory rehabilitation, Thoracoscopic esophagectomy.

INTRODUCTION

An esophagectomy with three-field lymph node dissection is the standard operation for esophageal cancer, and is performed widely in Japan. However, the effects of esophagectomy are still unsatisfactory compared with surgery for other gastrointestinal cancers, such as stomach, and colon cancer. Therefore, multidisciplinary therapy that uses a combination of radiation therapy and chemotherapy is recommended to improve the prognosis of patients with advanced esophageal cancer. The treatment of esophageal cancer has changed enormously over the past decade in Japan. Several new techniques have been

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introduced, and have contributed to the improved prognosis and results [1]. Based on these reports, we have introduced these new techniques into the treatment of esophageal cancer at our hospital.

In this paper, we describe three recently-introduced procedures for esophageal cancer at our hospital: thoracoscopic esophagectomy in the prone position, neoadjuvant chemotherapy, and perioperative care.

THORACOSCOPIC ESOPHAGECTOMY IN THE PRONE POSITION

Transthoracic esophagectomy is considered to be the most effective oncological operation in use throughout the world, however, complication rates have remained high, with a global inpatient mortality rate of about 10% [2]. The wound resulting from a thoracotomy and/or laparotomy can be problematic for patients, and is often associated with postoperative complications, including respiratory failure and pneumonia, which are mortality risks. To avoid such complications, several treatments, such as endoscopic mucosal resection, radiofrequency ablation, and definitive chemoradiotherapy, may be applied to patients previously considered as surgical candidates.

Minimally invasive techniques in surgery have been developed to reduce such complications. Cuschieri *et al.* reported on five patients that underwent thoracoscopic mobilization of the thoracic esophagus in 1992 [3]. Since then, various minimally invasive approaches techniques have been reported (Table **1**). We introduced Hybrid Minimally Invasive Esophagectomy (MIE) as thoracoscopic esophagectomy in 2008, whereby we performed thoracoscopic esophagectomy in the left lateral decubitus position. In 1994, Cuschieri *et al.* reported the first thoracoscopic esophagectomy in the prone position [5] and in 2006 Palanivelu *et al.* described their experience of MIE in the prone position [6]. In Japan, Noshiro *et al.* reported that the prone position provided a better view of the surgical field [7]. In reference to these reports, we changed the posture of the patient during the operation from the lateral decubitus position to the abdominal position in 2011.

During the three years from 2011 to 2013, we performed twenty-five esophagectomies for esophageal cancer, eight of which were performed

thoracoscopically in the prone position. In these cases, we experienced a small number of surgical complications, such as anastomotic leakage and stenosis, but the mortality rate was 0%, and all patients are still living.

Table 1. Variation of minimally invasive esophagectomy.

Total MIE (thoracoscopic and laparoscopic esophagectomy)
Hybrid MIE (either thoracoscopic or laparoscopic)
Laparoscopic-assisted transhiatal esophagectomy
Video-assisted mediastinoscopic transhiatal esophagectomy
Robot-assisted

Quoted from the paper of Watanabe *et al.* [4].
MIE, minimally invasive esophagectomy.

Jarral *et al.* described the benefits of prone esophageal surgery as including a shortened learning curve, ergonomic position of the hands, fewer thoracoscopic ports required, and no obstruction of the surgical field by the lung, blood or other factors [8]. Limitations include difficulty in the conversion to an open procedure and airway management [8]. We felt that the greatest advantage of using the prone position is a good field of vision for lymph node dissection at the superior mediastinum.

NEOADJUVANT CHEMOTHERAPY

Although surgery is a major modality in the treatment of esophageal cancer in Japan, many studies have been carried out to improve the prognosis on adjuvant therapy [9]. Until 2008, we mainly performed postoperative chemotherapy (cisplatin 70mg/m2 plus 5-fluorouracil 700mg/m2, continuous infusion on days 1-5 x 2 courses) as an adjuvant therapy in patients undergoing radical surgery for cancer in pathologic stage II or III.

In 2000, a high profile clinical trial began in Japan. In this study (JCOG9907, 2000-2006), patients with clinical stage II or III, squamous cell carcinoma, excluding T4, were randomly assigned to undergo surgery either followed or preceded by chemotherapy (cisplatin 80mg/m2 plus 5-fluorouracil 800mg/m2,

Part 1
TOPICS IN THE MODERN MEDICINE
B: NON-CANCER MANAGEMENT

CHAPTER 18

Adiponectin, Its Roles in Diabetes and Cardiovascular Disease

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Abstract: The recent increase in obesity is a worldwide social problem. Because obesity leads to the enormous increases in the patients with diabetes mellitus (DM) and coronary artery disease (CAD), it has become a growing health threat. Many researchers have found that various bioactive molecules released from adipose tissues, which are conceptualized as adipocytokines/adipokines, are involved in the pathogenesis of obesity-associated diseases. Adiponectin is an adipocytokine that exerts insulin-sensitizing effects in the liver and skeletal muscle and suppresses atherosclerosis in vascular walls *via* various anti-inflammatory effects. Clinically, circulating adiponectin levels inversely correlate with body mass index (BMI), which may explain the molecular basis that underlies obesity-associated development of DM and CAD. Many clinical studies have demonstrated that low circulating adiponectin levels are associated with the incidence of DM and CAD.

Recently, we showed that reduced adiponectin levels were associated with multi-vessel coronary artery atherosclerosis as detected on computed tomography coronary angiography (CTCA), and adiponectin may be a useful biomarker to predict multi-vessel coronary atherosclerosis when detected using CTCA in combination with common risk factors including age, sex, DM, and hypertension. Moreover, in another study, we showed that reduced adiponectin levels were associated with elevated serum oxidized low-density lipoprotein levels in which dyslipidemic components of metabolic syndrome were strongly involved. Thus, adiponectin levels are associated directly and

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indirectly with the development of atherosclerosis.

Measuring adiponectin concentration should provide beneficial information for the selection of high-risk patients and contribute to the reduction of fatal CAD by applying aggressive preventive therapy.

Keywords: Adiponectin, Diabetes mellitus, Metabolic syndrome, Obesity, Oxidized LDL.

INTRODUCTION

Based on physical inactivity and excess nutrition, the obesity rate worldwide is increasing and has become a social health problem because it is causally linked to a significant increase in the incidence of diabetes mellitus (DM). Specifically, visceral fat obesity leads to a clustering of cardiovascular risk diseases, designated as metabolic syndrome, which includes high fasting glucose, high triglycerides, low high-density lipoprotein (HDL) cholesterol, and high blood pressure. Metabolic syndrome leads to the development of DM and fatal cardiovascular diseases. Therefore, it is important to clarify the precise mechanism that underlies the obesity-associated development of these diseases, which will help establish effective strategies to improve the mortality and quality of life for obese individuals.

Recent studies have identified various types of bioactive molecules released from adipose tissue, termed as adipokines/adipocytokines. These include tumor necrosis factor alpha (TNFα) [1], interleukin-6 (IL-6) [2], monocyte chemoattractant protein 1 (MCP-1) [3], leptin [4], and adiponectin [5 - 11]. The discovery of adipocytokines led to researchers establishing the concept that adipose tissue is not merely an energy-storage organ but an endocrine organ that secretes many factors that contribute to glucose and lipid metabolism [12 - 14]. Furthermore, many studies have demonstrated that dysregulation of these adipocytokines contributes to the development of various obesity-related diseases.

Adiponectin is an endocrine factor released exclusively from adipose tissue [5, 8]. In obesity, especially visceral fat obesity, plasma adiponectin levels decrease [5]. Reduced adiponectin levels have been reported to be associated with DM and

coronary artery diseases (CAD) [15, 16]. Basic scientific studies have demonstrated that adiponectin has insulin-sensitizing [6, 7, 9], anti-atherogenic, and anti-inflammatory properties [10, 11]. Thus, adiponectin is recognized as one of the most important adipocytokines that contributes to the pathogenesis of DM and cardiovascular diseases.

ROLES OF ADIPONECTIN PROTEIN

Insulin-sensitizing Effects of Adiponectin

Various animal studies have demonstrated that adiponectin proteins have insulin-sensitizing or anti-diabetes effects. Adiponectin-deficient mice showed marked elevations in plasma glucose and insulin resistance when they were fed a high-fat and high-sucrose diet [9]. Adiponectin supplementation, *via* the transfection of an adiponectin-generating adenovirus, improved insulin resistance in these mice [9]. This insulin-sensitizing effect of adiponectin is mediated by the activation of adenosine monophosphate-activated protein kinase (AMPK) [17]. Moreover, adiponectin can facilitate fatty acid oxidization by activating peroxisome proliferator-activated receptors (PPAR) α [17]. These effects are mediated by the specific receptors of adiponectin, AdipoR1 and AdipoR2 [18]. Experiments using mice deficient in both AdipoR1 and AdipoR2 revealed that these proteins play crucial roles in mediating the insulin-sensitizing effects of adiponectin [19].

Anti-atherogenic Effects of Adiponectin

Adiponectin plays a protective role in the vascular wall from various processes that develop atherosclerosis (Fig. **1**). In an experimental vascular injury model, adiponectin-deficient mice developed more severe intimal thickening with more active smooth muscle cell proliferation compared to wild-type mice [10]. Treatment with adiponectin-producing adenovirus suppressed this intimal thickening [10]. Moreover, adiponectin adenovirus attenuated plaque formation in apolipoprotein E-deficient mice [11, 20]. *In vitro* experiments have demonstrated various anti-atherosclerotic effects of adiponectin. Specifically, adiponectin suppresses the expression of adhesion molecules, such as intracellular adhesion molecule 1, by inhibiting the TNFα-mediated activation of nuclear factor kappa B (NF-κB) in endothelial cells, which leads to the suppression of monocyte adhesion

CHAPTER 19

Novel Strategy for Treatment in Type 2 Diabetes Mellitus: Targeting Systemic and Adipose Tissue Inflammation

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Abstract: Type 2 diabetes (T2D) is a global health issue and developing new therapies continues to be urgent. Inflammation is thought to participate in the pathogenesis of obesity-induced insulin resistance and T2D, and is a potential target to treat this disease. Circulating white blood cell (WBC) number or C-reactive protein (CRP) concentration, which is a representative blood marker of the inflammation, can predict the incidence of T2D. Recent studies have shown that obesity is accompanied by chronic local inflammation in adipose tissue and increments of adipose tissue macrophage (ATM) number. ATMs are involved in the deterioration of systemic insulin sensitivity, and monocyte chemoattractant protein-1 (MCP-1) contributes to the migration of macrophages into fat and development of insulin resistance. Salicylate and thiazolidinedione (TZD) affect immune cells in circulating blood or adipose tissue, which leads to improved insulin sensitivity systemically. Pharmacological intervention for chronic inflammation may provide a new approach to the treatment of T2D in the future.

Keywords: Adipose tissue macrophage, Diabetes, Inflammation, Insulin resistance, Monocyte chemoattractant protein-1.

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INTRODUCTION

Over the past few decades, the number of type 2 diabetes patients has increased dramatically and is now recognized as a global health issue. A new breakthrough therapy is necessary to stop this growing diabetic trend and atherosclerotic complications. Obesity is a known risk factor for type 2 diabetes and chronic inflammation participates in the pathogenesis of obesity-induced insulin resistance and type 2 diabetes.

Type 1 diabetes is an autoimmune disease, and T cells, B cells, conventional dendritic cells (cDCs), plasmacytoid dendritic cells (pDCs) or neutrophils participate in the destruction of pancreatic beta cells [1]. Obesity and type 2 diabetes are also now considered systemic immunological diseases [2]. Many types of immune cells may play a crucial role in the development of obesity-induced insulin resistance. Macrophages, in particular, infiltrate into adipose tissue and are a major cause of obesity-associated chronic low-grade inflammation [3]. Recently, these inflammatory processes have been considered as a pharmacological target for insulin resistance and type 2 diabetes treatment [4].

INFLAMMATION MARKER PREDICTS TYPE 2 DIABETES

Activation of the immune system and inflammation can be quantified by increment of some circulating makers, including white blood cell (WBC) count, C-reactive protein (CRP), interleukin 6 (IL-6), or plasminogen activator inhibitor-1 (PAI-1). The number of circulating WBC is a predictor for developing type 2 diabetes [5, 6]. Interestingly, a high WBC predicts a worsening of insulin resistance, but it isn't associated with insulin secretion [6]. This data suggests that circulating WBC may be involved in the deterioration of insulin resistance, which develops into type 2 diabetes.

CRP is a representative marker of acute or sub-acute inflammation. Elevated levels of CRP were shown to predict the development of type 2 diabetes [7 - 9]. In a Cox proportional hazards model dividing quartiles of CRP, hazard ratios for diabetes development in the highest *versus* lowest quartile of CRP levels were 2.84 (95% CI 1.09 –7.39) among men and 3.11 (1.25–7.75) among women [9]. The other inflammation markers, IL-6, PAI-1, orosomucoid and sialic acid, were

also reported to be associated with developing type 2 diabetes [5, 7, 8].

PATHOGENESIS OF INFLAMMATION-INDUCED INSULIN RESISTANCE

What is the mechanism of inflammation-induced insulin resistance? One of the possible pathogenesis is that the pro-inflammatory cytokines disturb insulin signals. Hotamisligil *et al.* reported that neutralization of tumor necrosis factor-α (TNF-α) in obese rats rescued the obesity-induced worsening of insulin sensitivity [10]. This was the first evidence that the pro-inflammatory cytokine-induced serine phosphorylation of insulin receptor substrate (IRS) proteins underlies the inhibition of insulin signaling [10, 11].

Pro-inflammatory cytokines such as TNF-α and IL-1β activate JUN N-terminal kinase (JNK) and the inhibitor of the nuclear factor-κB (NFκB) kinase-β (IKKβ) signaling pathway in the liver, muscle and fat [12, 13]. Activation of the JNK pathway by these cytokines leads to serine phosphorylation of IRS proteins, which interferes with tyrosine phosphorylation of IRS and induces insulin resistance. In contrast, the activated IKKβ/ NFκB and JNK/ activator protein 1 (AP1) signaling pathway activates transcription of inflammatory genes, which may create a vicious cycle.

OBESITY-INDUCED IMMUNE-CELL INFILTRATION INTO ADIPOSE TISSUE

Energy excess leads to fat cell hypertrophy and larger adipocytes are associated with an insulin resistant state. A decade ago, two studies showed that obesity is accompanied by an increment of adipose tissue macrophage (ATM) number with larger adipocytes (Fig. **1**) [14, 15]. These two papers provided the initial evidence of macrophage involvement in obesity-induced adipose tissue inflammation and insulin resistance.

One of the candidates possibly involved in macrophage infiltration into adipose tissues is monocyte chemoattractant protein-1 (MCP-1). MCP-1 is a member of the CC chemokine family and promotes migration of inflammatory cells. Along with another group, we have shown that MCP-1 expression level in white adipose

CHAPTER 20

Improved Adaptation of Laparoscopic Partial Nephrectomy based on the Evaluation of Renal Function Using ^{99}mTc-MAG3 Renal Imaging

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Abstract: We evaluated renal function before and after laparoscopic partial nephrectomy by calculating differential renal function thru the use of renal scintigraphy with ^{99}mtecnetium-mercaptoacetyltriglysine (^{99}mTc-MAG3).

Differential renal function was assessed for 62 patients undergoing laparoscopic partial nephrectomy for renal tumors with renal scintigraphy before operation and six months post operation.

Mean operating time, ischemic time, blood loss and tumor diameter were 142 min, 27 min, 75 ml and 2.0 cm, respectively. The mean reduced rates of calculating glomerular filtration rate (cGFR) of a bilateral and the diseased-side kidney were 7.96% and 23.3%, respectively, and was statistical significant (p<0.001).

Meanwhile, the mean rate of increase of cGFR on the non-diseased-side kidney was 6.8%, which was significantly decreased (p=0.005). By univariate analysis, patients with a tumor of more than 2 cm in size and with clamping of the renal artery for over 30 minutes had decreased 6-month postoperative cGFR by more than 20%.

In multivariate analysis, tumor size was the most significant factor related to a decrease in cGFR. Patients with a tumor larger than 2 cm in size had a longer operation time (p=0.0005) and a higher amount of bleeding (p=0.0012).

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In comparison, the six-month postoperative cGFR of patients less than 55 years-old had significantly increased cGFR over 20%.

Lower cGFR of the diseased-side kidney after partial nephrectomy was predicted by longer ischemic interval and tumor size. Age predicted increasing cGFR of the non-diseased side after partial nephrectomy. Currently, we are endeavoring to shorten ischemic time. Younger patients with renal tumors should adapt to laparoscopic partial nephrectomy.

Keywords: Laparoscopic nephrectomy, MAG3, Partial nephrectomy, Renal cell carcinoma, Renal scintigraphy.

INTRODUCTION

Chronic kidney disease increases the risks of death, cardiovascular disease and use of specialized health care. Among a large, diverse population of adults, a reduced estimated glomerular filtration rate (eGFR) is associated with increased risks of death, cardiovascular events and hospitalization [1, 2]. Therefore, it is important to preserve renal function in the surgical treatment of renal tumor. Recently, the practice of nephron-sparing surgery has increased along with advancements in surgical techniques. Partial nephrectomy has been recommended for T1 renal cancers owing to advantages in improved postoperative renal function, equivalent oncological outcomes, and better overall survival compared with radical nephrectomy [3 - 5]. Since Winfield [6] first reported a partial laparoscopic procedure for renal tumors, it has become widely accepted for small renal tumors as the preferred surgical procedure and has oncologic outcomes similar to those of open partial nephrectomy and has decreased patient morbidity [7].

In the present study, we compared postoperative renal function six months after laparoscopic partial nephrectomy by a using a ^{99}mtecnetium-mercapto-acetyl-triglysine (^{99}mTc-MAG3) renal scintigraphy parameter (calculating glomerular filtration rate: cGFR), which enabled assessment of ischemic damage in isolation in the surgically preserved renal tissue after partial nephrectomy. We statistically evaluated factors that were influential for postoperative renal function compared with preoperative renal function.

MATERIAL AND METHODS

Patients

This was a retrospective study performed at the National Hospital Organization Kure Medical Center and Chugoku Cancer Center. From June 2010 until June 2013, 62 patients who had a renal tumor detected thru imaging and had a laparoscopic partial nephrectomy underwent ^{99}mTc-MAG3 renal scintigraphy prior to and six months after laparoscopic partial nephrectomy. To evaluate the functions of each kidney separately, we measured differential renal functions to calculate cGFR. The indication for laparoscopic partial nephrectomy was being distant to hilus and a size of less than 10 cm. A transperitoneal or retroperitoneal approach was adopted according to the location of the tumor. Just before operation, we inserted a 6Fr ureter catheter into the renal pelvis for perfusion of saline, which cooled the renal parenchyma and to inspect whether to open a renal collection system. 40 mg mannitol was injected intravenously before clamping and after unclamping the renal vessels. The renal arteries were clamped with bulldog forceps. If the tumor was close to a renal pedicle, we clamped the renal vein to prevent backflow hemorrhage from it. The transected collection system was repaired with a running 3-0 polyglactin suture. Renal parenchymal were sutured over oxidized regenerated cellulose bolster using 2-0 polyglactin. Recently, minor bleeding has been arrested by soft coagulation.

We analyzed influential factors in terms of postoperative renal function that were decreased by more than 20% for 6-month postoperative cGFR on the diseased side and increased by more than 20% for 6-month postoperative cGFR on the non-diseased side. The influential factors evaluated included tumor characteristics and surgical parameters such as age, BMI, tumor size, comorbidities (hypertension, diabetes hyperlipidemia and grade 3a-5 chronic kidney disease), RENAL score, ischemic time, bleeding volume and operation time. The RENAL nephrometry score was calculated according to previously published reports [8].

Multiple comparisons and multivariate analysis were used for multifactorial comparison. A p-value < 0.05 was considered significant.

CHAPTER 21

Recent Findings in Genetic and Enzymatic Analysis of Newborn Screening-Positive Subjects Based on Tandem Mass Spectrometry

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Abstract: Beginning 2014, all newborns living in Japan will have newborn screening (NBS) using tandem mass spectrometry (MS/MS). We participated in a pilot study for this new NBS initiative and provided diagnoses in cooperation with Hiroshima University and other facilities. Here, we introduce the information that we obtained. Medium-chain acyl-CoA dehydrogenase deficiency (MCADD), ACADM mutations found among Japanese patients are quite different from those of Caucasian patients. We estimated MCAD activities of 11 mutants found among Japanese patients and positively screened babies using molecular genetics methods. Some mutations were destructive, but others found by NBS maintained activities to a certain degree. Regarding methylmalonic academia (MMA), we found the first Japanese patient with isolated methylmalonic acidemia caused by a cblD defect. Unfortunately, he was negative in the NBS and developed acute metabolic decompensation during an acute illness. We found MMADHC mutation heterozygously in this patient. Through our study, we offered reliable data for most of the positively screened newborns and their family members through our original enzymatic assay using peripheral blood mon-

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onuclear cells. We also obtained beneficial information from these investigations. In order to raise the quality of the newly introducing MS/MS-NBS system in Japan, it is essential to achieve accurate diagnosis through a combination of enzymatic and genetic evaluation.

Keywords: Medium-chain Acyl-Coa dehydrogenase deficiency, Methylmalonic academia, Tandem mass spectrometry, Newborn screening.

INTRODUCTION

In 1977, the Ministry of Health and Welfare (MHW) Japan initiated publicly funded newborn screening (NBS) for five congenital metabolic disorders. In recent years, NBS has included six disorders (congenital adrenal hyperplasia, congenital hypothyroidism, homocystinuria, maple syrup urine disease, galactosemia and phenylketonuria). Through NBS screening, more than 10,000 babies were saved from impediments.

From the 1990s, tandem mass spectrometry (MS/MS) was introduced for NBS in western countries. This new technology enables screening of newborns for over twenty disorders using only a few drops of the baby's blood. In 1997, MS/MS was introduced to NBS as a pilot study in several areas of Japan [1]. This has continued through the last decade, with the participation rate gradually increasing up to one fifth of all births per year.

Recently, the MHW notified all local governments in Japan to introduce MS/MS based NBS for 16 disorders. From 2014, all newborns living in Japan will have this new NBS. Despite the expected significant increase in examinees, facilities that are able to provide detailed investigations and diagnosis are currently limited in Japan.

We have been participating in the pilot study mentioned above and made diagnoses in cooperation with Hiroshima University and other facilities. Specifically, we have evaluated residual enzyme activities and analyzed genes responsible for suspected disorders for newborns with positive screens and of family members who were suspected to be carriers [2]. Here, we introduce the information that we have obtained.

EVALUATING ACTIVITIES OF MEDIUM CHAIN ACYL-COA DEHYDROGENASE DEFICIENCY (MCAD) MUTANTS FOUND AMONG JAPANESE PATIENTS

To date, over twenty Japanese patients with medium-chain acyl-CoA dehydrogenase deficiency (MCADD) have been found. Recently, c.449-452delCTGA (449del4) mutation was reported as a common mutation [3]. The other mutations are unique and uncharacterized. To characterize these mutants, we performed analysis of their functions using a gene expression system.

Eleven patients with MCADD were studied. Five were diagnosed after metabolic decompensation and six were detected from the NBS. We generated eleven mutations found among them by mutagenesis. Plasmid DNAs carrying the WT or each mutant were introduced into HEK293 cells. We evaluated n-octanoyl-CoA dehydrogenase activity of crude cell lysate including mutant MCADD protein by measuring 2-octenoyl-CoA formation at 37°C.

Six mutants showed less than 10% residual activity. In comparison, five showed over 50% activity. We examined residual activities of the later five mutants at a higher reaction temperature, and found that one showed a significant decline. These five mutants were detected from the NBS and from slight symptoms.

Our results suggest that some of those newborns detected from the NBS could have high residual enzyme activity. Detailed genetic and enzymatic evaluation is essential for appropriate follow-up of cases suspected for MCADD by MS/MS-NBS in Japan.

DETECTION OF THE FIRST JAPANESE PATIENT WITH ISOLATED METHYLMALONIC ACIDEMIA CAUSED BY A CBLD DEFECT

The prevalence of methylmalonic academia (MMA) in Japan has been estimated as 1/110,000, based on our neonatal-screening pilot study. In regard to MMA caused by adenosylcobalamin deficiency, only cblA defects have been identified. Here, we report the first Japanese case of a cblD-MMA patient.

A one-year-old boy developed disturbance of consciousness accompanied by severe metabolic acidosis and mild hyperammonemia during noroviral

CHAPTER 22

The Role of an Expert in Medical Engineering in Japan

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Abstract: Recent advancements in medical technology have led to development of various medical equipment and have improved clinical outcomes. Management of medical equipment requires expertise and knowledge of technical know-how, and there is a growing need for professional engineers in clinical practice. In Japan, a clinical engineer (CE) operates life-sustaining devices and provides maintenance management of life-sustaining device; the term CE was officially established in 1987. CEs have contributed to the development of a medical procedure by becoming technical experts.

In our hospital, the hyperbaric oxygen chamber operated by a CE was used to treat patients diagnosed with sudden sensorineural hearing loss or decompression illness. This job requires expertise of target illnesses. The oxygenator for cardiopulmonary bypass surgery is also operated by a CE, and handling of this instrument needs an inventive approach, as it directly affects the surgical outcome. For implantable cardiac

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devices, the CE helps to merge knowledge from the fields of electrophysiology and engineering. Configuring accurate device programming improves patients' prognoses. Furthermore, not just life-sustaining devices but also various medical equipment are centrally managed by the CE. Such management allows efficient use of the medical equipment.

In Japan, the CE contributes tremendously to clinical practice. CEs make the efficient use medical procedures using inventive approach or by active participation or management of these devices.

Keywords: Cardiopulmonary bypass, Clinical engineering, Hyperbaric oxygen therapy, Implantable cardiac device.

INTRODUCTION

With advancements in medical technology, various medical equipment have been developed to improve clinical outcome. The management of such medical equipment requires relevant expertise and knowledge of technical know-how, and therefore, professional engineers are required in clinical practice. In Japan, a clinical engineer (CE) operates and maintains the management of life-sustaining devices; the term CE was officially established in 1987. CEs contribute to the development a medical procedure by providing their expertise.

Hyperbaric oxygen chamber and heart-lung equipment are usually operated by the CE. For management of these machines, the CE requires to have knowledge of the target illness and the technique used for treating the target illness. For implantable cardiac devices, a widely used device, the CE helps to merge knowledge from the fields of electrophysiology and engineering. Furthermore, in addition to life-sustaining devices, various other medical equipment are also centrally managed by a CE.

Here, we review the findings of our recent research for improving our clinical performance.

HYPERBARIC OXYGEN THERAPY

Our hospital owns a multiple hyperbaric oxygen chamber that is operated by a CE. Generally, patients who are diagnosed with conditions such as sudden

sensorineural hearing loss (SSHL), facial paralysis, decompression illness, and carbon monoxide poisoning receive hyperbaric oxygen therapy. Notably, in our hospital, 70% of patients who undergo hyperbaric oxygen therapy are those with SSHL.

We retrospectively evaluated the appropriate therapeutic period in patients with SSHL who received hyperbaric oxygen therapy. The patients were categorized into effective and ineffective groups, depending on whether the treatment was effective [1]. Table **1** shows that, in the effective group, the mean audiometer value, which is indicative of hearing ability, improved significantly after 1-week treatment compared to that initially, and that hearing ability further improved after a 2-week treatment compared to that at 1 week; however, there was no further improvement after 3, 4, and 5 weeks of treatment. In the ineffective group, there was no improvement at any time point. The observation here is similar to that in a previous study [2]. Therefore, we concluded that the therapeutic effectiveness of hyperbaric oxygen therapy in patients with SSHL should be determined within 2 weeks. Patients diagnosed with SSHL should be administered simultaneous medication therapy [3, 4]. However, our study had a limitation in that, we could not explain the difference in the significant effect between hyperbaric oxygen therapy and medication therapy. Some researchers [5, 6] reported that the addition of hyperbaric oxygen therapy to conventional medication therapies was more effective than medication therapy alone in patients younger than 50 years.

Table 1. Audibility data measured per week using an audio-meter in patients diagnosed with sudden sensorineural hearing loss. Mean audibility data after 2, 3, 4, and 5 weeks of treatment are compared to those at 1 week by using Student's t-test.

Group	Day after the first therapy	n	Hearing ability (dB) Present		Hearing ability (dB) One week ago		P(t)
			mean	SD	mean	SD	
Effective	1 week	87	35.8	22.8	56.3	25.9	<0.001
	2 week	66	27.2	16.0	41.0	23.6	<0.001
	3 week	27	29.1	15.1	36.8	18.1	0.103
	4 week	15	34.4	14.5	36.9	15.4	0.663
	5 week	7	34.3	17.0	38.4	15.8	0.673
Ineffective	1 week	202	53.7	28.2	56.3	27.7	0.355
	2 week	160	52.4	26.9	54.9	28.7	0.414
	3 week	61	52.1	22.2	54.2	23.4	0.619
	4 week	12	55.7	15.4	59.1	14.8	0.606
	5 week	3	60.0	9.4	55.4	10.8	0.675

Abbreviations: dB, decibel; P(t): p-value by Student's *t*-test; SD, Standard deviation

Part 1
TOPICS IN THE MODERN MEDICINE
C: DEPRESSION AS A TARGET OF THERAPY

CHAPTER 23

Depression: A Novel Mechanism of Antidepressant Action with a Focus on Astrocytes

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Abstract: Postmortem studies of patients with depression show a reduction of glial cells and altered expression of glia-related genes in restricted areas of the brain, suggesting that glia are part of a neural network affected in the pathophysiology and treatment of the disorder. Glia, especially astrocytes, are major components in the CNS and play a role in the storage of several types of neurotrophic factors that might be systematically associated with the pathophysiology and treatment of depression. The expression of neurotrophic factors caused by antidepressants in glia is regulated by not only a monoamine-dependent, but also a monoamine-independent mechanism. Our data demonstrated that astrocytes stimulated by an antidepressant may be important mediators that produce several neurotrophic/growth factors, especially GDNF and FGF-2, through a *de novo* protein synthesis-dependent and a monoamine-independent mechanism. Antidepressants act directly on astrocytes to increase GDNF production after the activation of the FGFR/FRS2alpha/ERK/CREB signaling cascade *via* a MMP-dependent shedding of FGFR ligands. Clarifying monoamine-independent novel targets of antidepressants in astrocytes may contribute to the development of more effective treatments for depression.

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Keywords: Antidepressant, Astrocyte, Depression, Glia, Neurotrophic factor.

INTRODUCTION

In the human brain, glial cells are estimated to exist in almost the same number as neurons. Recent evidence has indicated that the active brain should be a circuit of integrated interactive neurons and glial cells. To understand the underlying biological mechanisms of psychiatric disorders or the actions of antidepressant therapy, glial cells should receive much greater attention [1]. In particular, post-mortem reductions in glial cell numbers in these regions and more subtle changes in neuronal density and size in patients with mood disorders have been reported [2]. The rodent model, which is transiently disturbed in the glia of the prefrontal cortex by specific chemical agents, shows depressive-like behavior, supporting the findings of postmortem studies [3, 4]. In comparison, neurotrophic/growth factors are directly involved in maintenance of the function and structural integrity of neurons in the adult brain [5]. Abnormalities in neurotrophic/growth factors may be implicated in the etiology of mood disorders [6 - 8]. These findings allow for the possibility that both dysfunction of glia and abnormalities in neurotrophic/growth factors may be tightly associated with each other in mood disorders. This review focuses on glial cells, especially astrocytes, and neurotrophic/growth factors in mood disorders and their treatment with antidepressants.

POST-MORTEM BRAIN STUDIES IN MOOD DISORDERS

Post-mortem histological analyses have reported reduced glial cells in number and density in pyramidal layers III and V of the frontal cortical areas in major depressive disorder (MDD) and bipolar disorder (BD). In MDD, a decreased density of Nissl-stained glial cells was observed within layers III and V of the dorsolateral prefrontal cortex (Broadman area 9) in comparison to control subjects [9]. In addition, glial cells were reduced in number in mood disorders in other areas of the frontal cortex including the orbital prefrontal cortex [10], and the anterior cingulated cortex [11]. Importantly, in the frontal cortex in MDD, expression of glial high-affinity glutamate transporters, SLC1A2 and SLC1A3, and L-glutamate-ammonia ligase which converts glutamate to nontoxic glutamate

in astrocytes, were significantly decreased, suggesting a dysfunction of glutamatergic and GABAergic systems through glia in MDD [12].

Astrocytes may be one of the glial cell types that are involved in the changes of glial cell numbers in MDD. Astrocytes are classically identified histologically by their expression of GFAP. Although reduced glial activity with no substantial changes in the numbers of astrocytes cannot be ruled out, a decline in the numbers of astrocytes in the frontal cortex in MDD, as mentioned in the previous paragraph, may contribute to the reduced GFAP levels. Several studies have reported that alteration of immunoreactive astrocytes and the expression of GFAP in density has been reported in the frontal cortex in MDD [13 - 15]. A proteomic analysis of brain tissue from MDD patients showed reductions in four isoforms of GFAP in prefrontal area 10 [16].

The reduction in glial cells should be more consistent than the pathology of neuronal cells in areas of the prefrontal cortex in patients with MDD, although there are a few reports of neuronal change in GABAergic interneurons [17]. Actually, animal studies have revealed that chronic psychosocial conflict can result in fewer astrocytes, and fluoxetine treatment can block this effect of long-term stress [18]. In contrast, in the rodent model, which is transiently disturbed by specific chemical agents in the glia of the prefrontal cortex, a depressive-like behavior is demonstrated [3, 4]. These changes of astroglial structural plasticity in response to stress and antidepressant treatment support the finding that glial changes might contribute to the pathophysiology of mood disorders and the cellular actions of antidepressants.

GLIA AND NEUROTROPHIC FACTOR

Glial cells synthesize and release many neurotrophic factors, growth factors, and cytokines vital for neuronal health. They are nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and ciliary neurotrophic factor (CNTF) [19]. The mechanisms controlling the release of trophic factors are poorly understood. As a model, trophic factors produced and released by glia such as astrocytes act on

CHAPTER 24

Advances in Electroconvulsive Therapy for Psychiatric Disorders

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Abstract: We reviewed the history of electroconvulsive therapy (ECT) around the world and in Japan. Although the practice of ECT began in Japan at around the same time as the discovery of ECT in the world, improvements in anesthetics and ECT devices in Japan have not progressed at a similar pace in comparison with that of the world. Recently, a brief pulse device was approved for use in Japan and ECT practice guidelines were established. ECT has been recently reevaluated and an ECT network has been extended in Japan.

Specifically, we offer here a history of ECT and clinical and biological research findings for ECT from the Kure Medical Center. In 2013, our center conducted nearly 500 ECT sessions with approximately 40 individuals who had mood disorders, schizophrenia or Parkinson disease. Research on ECT has shown that several factors are important in recovery. For example, a strategy that includes a dosage of stimulation energy or reduction of anesthetic might be effective for ECT treatment. Mood stabilizers such as lithium may be effective in preventing relapse after ECT, not only in mood disorders, but also for schizophrenia. In addition, cerebral blood flow evaluation using near-infrared spectroscopy and blood markers such as matrix metalloproteinases may be related to diagnosis and the course of ECT treatment.

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Keywords: Biological research, Clinical research, Electroconvulsive therapy, Guideline, History.

HISTORY OF ECT IN THE WORLD

Electroconvulsive therapy (ECT) is a psychiatric treatment in which electric currents are passed through the brain, intentionally triggering a brief seizure. In 1938, Italian neuropsychiatrists Drs. Cerletti and Bini demonstrated ECT in Rome for the first time [1]. The popularity of ECT spread among psychiatrists as a form of treatment for severe mental diseases in the 1940s and 1950s; use of ECT declined in the 1970s and 1980s after the introduction of pharmacotherapy for severe mental disorders [2]. The negative depictions of ECT in mass media also contributed to its decline. During this time, the main indications for ECT transformed from first-line to last-resort treatment for medication-resistant and very severe life-threatening clinical conditions [2, 3]. However, in 2001, the American Psychiatric Association (APA) developed guidelines advising that ECT should not be used only as a last resort.

In the beginning, ECT was usually given in an unmodified form, without muscle relaxants, and the seizure resulted in a generalized tonic-clonic convulsion. A rare, but serious, complication of unmodified ECT was fracture of the backbone. In 1952, Drs. Holmberg and Thesleff modified ECT and used it in combination with succinylcholine, as a muscle relaxant, and barbiturates [4]. Several guidelines have recommended modified ECT as the standard routine [5, 6].

In the 1980s, evidence indicated that sine-wave currents lead to greater cognitive side effects with no advantages compared to brief pulse square waveforms [7]. Brief pulse machines slowly replaced the sine-wave devices, and are now advised as the standard treatment [5, 8].

HISTORY OF ECT IN JAPAN

In 1939, at almost same time as foreign countries, Drs. Yasukouchi and Mukasa who belonged to Kyushu University in Japan successfully used ECT to treat patients with schizophrenia [9]. Dr. Shimazono of Tokyo University reported a modified version of ECT in 1958 [10]; however, this version did not become

widespread in Japan most likely because little collaboration occurred between psychiatrists and anesthesiologists at that time.

In 1993, Dr. Nakashima investigated the present condition of ECT for members of the Japanese Society of Psychiatry and Neurology (JSPN) in which he described that the modified ECT was used for only approximately 20% of all ECT-treated patients [11]. In 2002, the brief pulse device (Thymatron, Somatics, Lake Bluff, IL, USA) was officially approved for use in Japan [12]. Again, a study of ECT practice in Japan from 2001 to 2003 was conducted among 100 institutes of psychiatry [13]. Even at that time, Japanese psychiatrists were not familiar with ECT, and over half of cases in which ECT was administered were unmodified [13]. At present, ECT practice has changed among universities and general hospitals to the modified ECT protocol that uses the brief pulse device [14]. The effectiveness of ECT has also been also been reevaluated, especially for the treatment of depression.

In 1998, the Japanese Society of General Hospital Psychiatry (JSGHP) began establishing ECT guidelines [14]. In 2001, the ECT committee of JSPN made a tentative draft of the ECT guidelines. In 2002, this committee translated "*A task force report of the APA: the practice of electroconvulsive therapy*" in Japanese to expand the use of ECT [15]. In 2005, the ECT committee of the JSPN made recommendations for performing ECT that conformed to the APA guidelines and emphasized both the use of the modified ECT protocol with the brief pulse device and the thoroughness of obtaining informed consent. In 2008, the ECT committee of the JSPN conducted a survey of ECT practice to reexamine its use and establish guidelines for Japanese psychiatrists based on a direct recommendation from the APA to improve its methodology for performing ECT because many institutions continued using the unmodified ECT in Japan. Subsequently to the history of ECT practice and guidelines in Japan, "*Recommendations for ECT Practice, Second Edition*" was published in 2013 [16]. For the purpose of sharing common experiences with ECT and contributing to a reexamination of the ECT guidelines, the ECT network meeting in metropolitan area has been held a meeting from February 2009. This meeting sought to establish a local ECT network to be located in prefectures such as Hiroshima. At present, the expanded application of ECT is eagerly anticipated in Japan [14].

CHAPTER 25

How Does Electroconvulsive Therapy Work in the Brain? –Involvement of the Astrocyte-Derived Synaptogenic Factor, Thrombospondin-1-

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Abstract: Antidepressants and electroconvulsive therapy (ECT) are major therapeutic strategies for mood disorders. ECT is the most potent treatment for antidepressant-resistant mood disorders; however, the underlying mechanisms of action remain largely unclear. Therefore, the identification of the molecular and cellular mechanisms affected by ECT may provide further insight into the pathophysiology of depression and the development of more effective therapeutic strategies.

Herein, a variety of hypotheses on the pathophysiology of mood disorders and the mechanism of antidepressive treatments are reviewed, with an emphasis on synaptogenesis. Our findings suggest that synaptogenesis is involved in the mechanism of action of ECT, possibly *via* thrombospondin (TSP)-1, a member of TSP family that was reported to be secreted by astrocytes to regulate synaptogenesis in the brain.

Keywords: Astrocyte, Electroconvulsive therapy, Mood disorder, Synaptogenesis, Thrombospondin-1.

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PATHOPHYSIOLOGY OF MOOD DISORDERS AND MECHANISM OF ACTION OF TREATMENT STRATEGIES

Mood disorders are a major class of psychiatryic disorders with a lifetime prevalence of 6.5% in Japan: one in every fifteen individuals will suffer from a mood disorder at least once during their life.

Previous research on the pathophysiology of depression and the mechanism of action of antidepressive treatments have focused on monoamine, since traditional antidepressants, such as tricyclic antidepressants, commonly affect monoamine reuptake. However, several therapeutic phenomena remain unexplained. For example, the effects of antidepressants take several weeks to manifest and some patients do not respond to antidepressants at all. Therefore, monoamine alone may not fully explain the pathophysiology of depression and the mechanism of action of antidepressive treatments.

Recent advanced imaging and post-mortem studies have revealed a reduction in the total volume and cell density and size of particular areas of the brain, including the prefrontal cortex, hippocampus and amygdala, in patients with mood disorders [1, 2]. In contrast, it was reported that antidepressants and electroconvulsive seizure (ECS), an animal model of ECT, increased neurotrophic/growth factors such as brain-derived neurotrophic factor (BDNF) in the brains of animals [3]. Therefore, neurotrophic factors have become the focus of a leading hypothesis which suggests that depression is associated with a loss of neural and glial plasticity and that neurotrophic factors are produced to repair the abnormality and bring about antidepressive effects.

In the frontal cortical area, a reduction in glial cell number and density has been reported in mood disorders [4, 5]. In the human brain, the number of glial cells exceeds the number of neurons in the cerebral cortex, although the number of glial cells is similar to that of neurons in the brain as a whole [6]. In addition, the glia is known to play a major role in the production of neurotrophic/growth factors in the brain. In addition, an animal study where astrocytes were pharmacologically ablated suggested that the loss of astrocytes is sufficient to induce depressive-like behavior [7]. Therefore, the glia has become a novel target of research into the

pathophysiology of mood disorders and the effects of antidepressants [8].

Quite recently, synaptic dysfunction has received attention for its involvement in mood disorders [9]. Indeed, the number of synapses has been reported to decrease in certain brain regions in depressed patients [10]. Furthermore, the dysregulation of synaptic genes has also been reported in patients with major depressive disorder (MDD) [10 - 12]. Ketamine, a fast-acting and effective agent in MDD patients resistant to traditional antidepressants, induces synaptogenesis and reverses the synaptic deficits caused by chronic stress [13]. ECS has been reported to increase the total number of synapses in the adult rat hippocampus [14]. Hippocampal synaptogenesis is one theory on the mechanism of action of ECT works [15]; however, the precise underlying mechanism remains unclear.

SYNAPTOGENESIS AND ASTROCYTE-SECRETED SYNAPTOGENIC FACTORS

As indicated in Fig. (**1**), synaptogenesis comprises three steps: 1) induction of ultrastracturally normal synapses; 2) maturation of the presynaptic terminal (vesicle cycling at the presynaptic terminal); 3) maturation of postsynaptic activities (glutamate receptor insertion at postsynapse) [16].

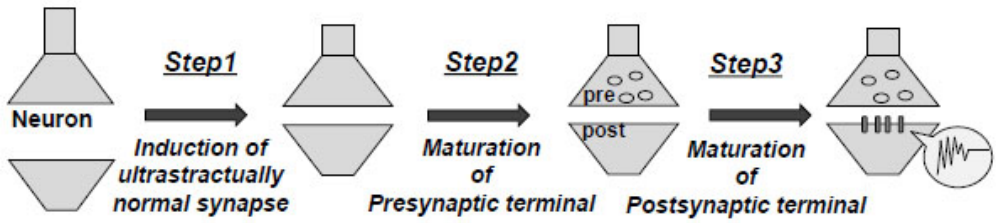

Fig. (1). Schematic depiction of the mechanism of synaptogenesis. Synaptogenesis is composed of three steps as indicated above.

Thrombospondin (TSP)-1 and -2 are the first discovered astrocyte-secreted proteins which induce synaptogenesis in the brain [17]. TSP-1 and -2 play an essential role in step 1 and step 2, however these synapses are postsynaptically silent (Table **1**) [17]. Most recently, Allen *et al.*, have identified glypican-4 and -6 as astrocyte-secreted factors that are involved in step 3 (Table **1**) [18]. Furthermore, hevin and secreted protein acidic and rich in cysteine (SPARC) have been identified as other

Part 1
TOPICS IN THE MODERN MEDICINE
D: EXPERIENCE IN NATIONAL DISASTER

CHAPTER 26

Response to the Great East Japan Earthquake: Medical Aid Activities by National Hospital Organization

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Abstract: Japan's National Hospital Organization (NHO), a Designated Public Corporation specified in the Disaster Countermeasure Basic Act, promptly responded to the 2011 Great East Japan Earthquake by mobilizing medical teams from NHO hospitals nationwide. This report describes the activities conducted by NHO and lessons learnt for better preparedness and response in the future.

Keywords: Disaster, Disaster medical assistant teams, Earthquake, Mobile medical teams, National Hospital Organization.

THE NHO'S ROLE AND RESPONSIBILITY IN JAPAN'S FRAMEWORK OF DISASTER MANAGEMENT

The Disaster Countermeasures Basic Act (enacted in 1961) specifies 56 Designated Public Corporations, such as the Bank of Japan, Electric Power Companies and Nippon Telegraph, and Telephone Corporation. The National Hospital Organization (NHO) is one of the two Designated Public Corporations specializing in the medical field. The Act stipulates that each Designated Public Corporation shall formulate and implement its own Disaster Management Operation Plan.

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The NHO Disaster Management Operation Plan includes the establishment of a Disaster Response Headquarters, the designation of disaster base hospitals and organization and dispatch of mobile medical teams. (As of April 2014, 34 of Japan's 143 NHO hospitals were designated).

On 11 March 2011, the Great East Japan Earthquake, the largest earthquake ever recorded in Japan struck the northeastern coast of Honshu. The ensuing tsunami devastated cities and towns in the region [1, 2]. Struggling with disrupted power and water supplies and communication systems, NHO hospitals in the affected region admitted patients needing emergency care. Patients were also transferred from other hospitals that had more serious damage.

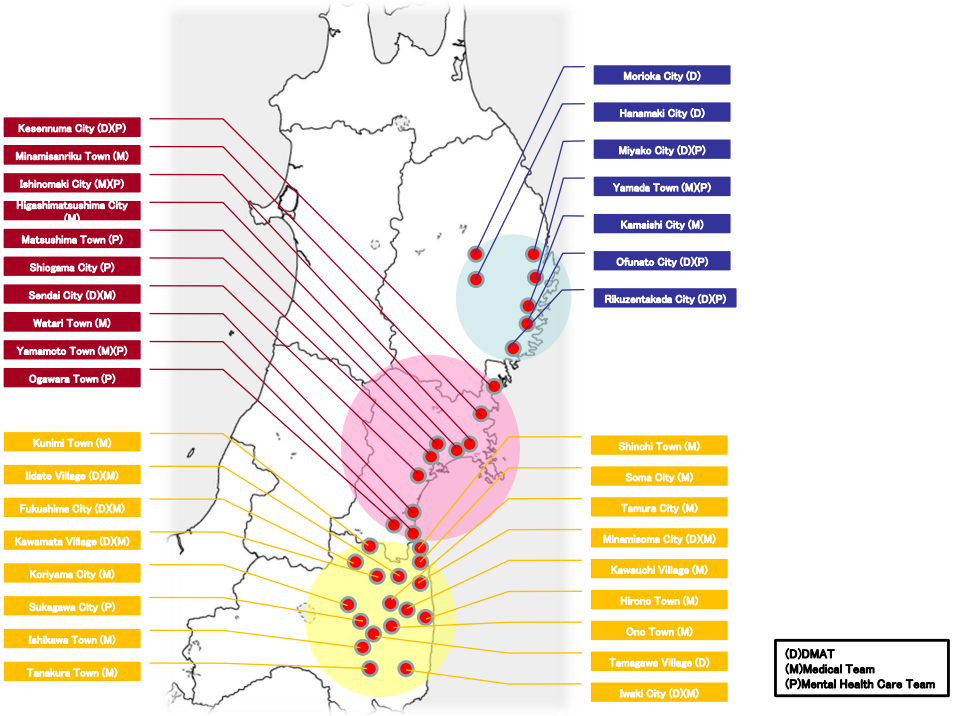

Fig. (1). Districts where NHO hospitals dispatched assistance teams in response to the Great East Japan Earthquake.

A Disaster Response Headquarters, headed by the President of the NHO, was immediately set up. It collected and analyzed information on the impact of the disaster, and decided policies on medical operations. An Onsite Disaster Response

Headquarters was then created and staffed with people from NHO headquarters. The Disaster Response Headquarters decided to send mobile medical teams from NHO hospitals outside of the disaster-stricken areas (Figs. **1** and **2**).

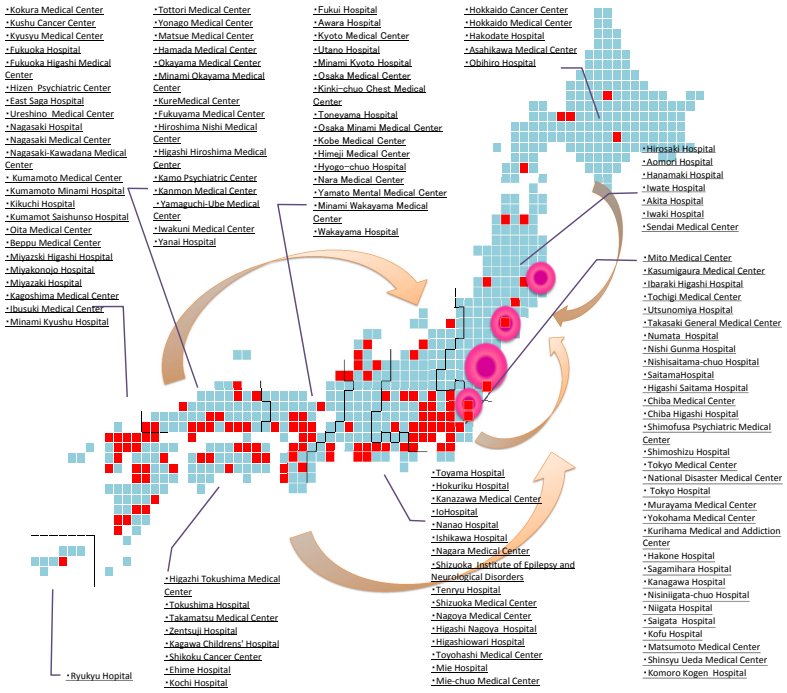

Fig. (2). NHO hospitals which dispatched personnel as DMATs, Mobile Medical Teams, Mental Health Care and other form of assistance.

COORDINATION AND DISPATCH OF JAPAN DISASTER MEDICAL ASSISTANT TEAMS

Taking into account the lessons learnt from the 1995 Great Hanshin Awaji Earthquake, Japan Disaster Medical Assistant Teams (DMAT) were established. DMAT is defined as a trained, mobile medical team that can be deployed in the acute phase of a disaster (within 48 hours of a disaster). DMAT provides medical treatment in a staging care unit and transports severely injured or ill patients from the disaster-affected areas. The DMAT secretariat is located within the NHO National Disaster Medical Center. The Emergency Medical Information System (EMIS) was also established after the Great Hanshin Awaji Earthquake to share information on the impact of disaster and activities of DMAT in real time.

Part 2
ADVANCES IN THE MODERN MEDICINE

CHAPTER 27

The Yin and Yang of Von Willebrand Factor in Thrombosis and Hemostasis: Lessons from Von Willebrand Disease and Thrombotic Thrombocytopenic Purpura

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Abstract: Various factors, including platelets, endothelial cells, and coagulation factors, regulate the mechanisms of thrombosis and hemostasis. Von Willebrand factor (VWF) is one of the key molecules to initiate platelets adhesion and aggregation. Quantitative and qualitative VWF abnormalities cause both hemorrhagic and thrombotic disorders. The role of VWF has emerged from molecular mechanisms of von Willebrand disease (VWD) and thrombotic thrombocytopenic purpura (TTP). This review summarizes the current knowledge of these diseases and the importance of VWF in thrombosis and hemostasis.

Keywords: ADAMTS13, Hemostasis, Thrombosis, TTP, Von Willebrand factor.

VON WILLEBRAND DISEASE

Von Willebrand disease (VWD) is an inherited bleeding disorder that was first reported by Eric von Willebrand in Finland. In 1926, he described that this disor-

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der differs from hemophilia in several aspects, including 1) mucocutaneous bleeding rather than bleeding in joints and muscles, 2) autosomal inheritance rather than recessive inheritance linked to the X chromosome, 3) elongation of bleeding times, and 4) normal clotting time [1]. Later, VWD was defined as qualitative or quantitative abnormalities of von Willebrand factor (VWF) [2 - 4].

1. Structure, Synthesis and Cleavage of Von Willebrand Factor

VWF has various functions, 1) VWF acts as the carrier of plasma coagulation factor VIII (FVIII). 2) VWF is required for the adhesion of platelets to collagen on the injured sub-endothelium. 3) VWF is also required for platelet-to-platelet adhesion and aggregation [5]. VWF protein is first synthesized as a large protein consisting of 2813 amino acids [6]. The signal peptide (aa 1-22) and the propeptide (aa 23-763) are sequentially cleaved. The mature VWF subunits (aa 764–2813) were assembled into linear strings called "VWF multimers". Depending on the numbers of subunits, the sizes of multimers vary, with larger multimers having stronger activities as adhesion molecules [5].

VWF is synthesized in two types of cells. In endothelial cells, VWF is synthesized and stored in Weibel-Palade bodies [2]. In megakaryocytes, VWF is also synthesized and stored in platelet alpha-granules [7]. VWF is released from these storage granules by vascular injury or platelet activation, and a conformational change of VWF multimer is induced by the high shear rates at the injured vessel wall. VWF multimers can then work as adhesion molecules on exposed subendothelium and platelet membranes.

As described later in this review, the VWF cleaving protease was identified and named as ADAMTS13 (adisintegrin and metalloproteinase with thrombospondin type 1 motives 13) [8]. Under high shear rates, plasma VWF multimers are subjected to physiologic degradation by ADAMTS13.

2. Classification of Von Willebrand Disease

VWD is classified into two distinctive quantitative types (type 1 and 3) and one qualitative type (type 2) [2 - 4].

VWD Type 1 and 3: Quantitative Types

Type 1 patients show relatively mild bleeding tendency because of a partial quantitative deficiency. Type 3 patients show severe bleeding tendency, as this type is characterized by the absence or only trace amounts of VWF. In type 3, FVIII levels are also lower because VWF acts as carrier protein of FVIII.

VWD Type 2: Qualitative Type

Type 2 VWD missense mutations cause functional abnormalities in VWF molecule and cause qualitative abnormalities [9 - 11]. In fact, the analysis of abnormal molecules of these subtypes clarified most of the different functions of each domain of VWF (Fig. **1**) [4, 6].

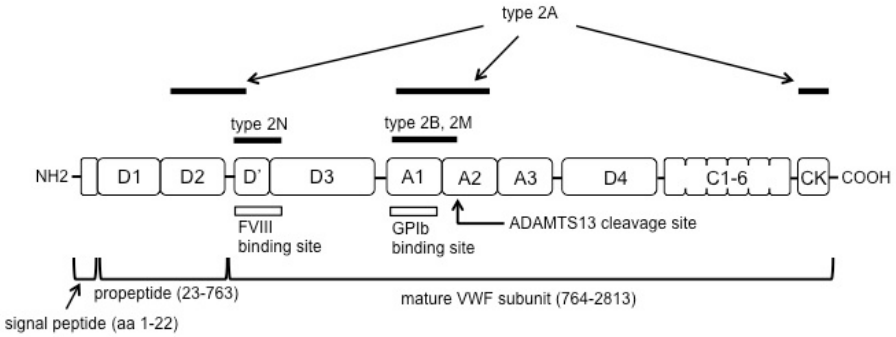

Fig. (1). Structure of VWF and location of VWD mutation showing the revised annotation of the VWF domain structure [4]. Black lines indicate locations of VWD mutations. White lines indicate locations of binding sites of factor VIII and GPIb.

Type 2A is the most common subtype of type 2 and shows the lack of large multimers. As the large multimers have stronger adhesion activities, the lack of large multimers induce bleeding tendency. Through the molecular analysis, a series of mutations were identified at the VWF propeptide, D/D3 and CK domains [4]. These mutations affect primarily multimer assembly, and indicating the importance of this domain for multimer formation. The other mutation cluster was identified around the A2 domain, and these mutations interfere with the folding of the A2 domain correctly [9, 12]. These abnormal A2 domains are accessible to ADAMTS13 even in the absence of high shear rates. Furthermore, proteolysis

CHAPTER 28

Topics in Neurology

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Abstract: Over the last decade there have been many advances in neurology. This article reviews topics in neurology, specifically for stroke and neurodegenerative diseases. Advances in magnetic resonance imaging provide a powerful tool for diagnosing stroke and thrombolysis by alteplase and have facilitated a paradigm shift in the approach to super-acute phase ischemic stroke. Secondary prevention of ischemic stroke has changed in terms of both anti-platelet therapy and anti-coagulation therapy. Over the last decade, understanding about the etiology and pathogenesis of many neurodegenerative diseases has progressed. Recent reports are reviewed here regarding Alzheimer's disease, Parkinson disease, dementia with Lewy bodies, motor neuron disease and frontotemoral dementia as they share many common mechanisms involving aggregation and accumulation of proteins.

Keywords: Neurocognitive disorders, Neurodegenerative diseases, Novel oral anticoagulation agents, Stroke, Thrombolysis.

STROKE

The slogan for World Stroke Day designated by the World Stroke Organization (WSO) is, "One in six people worldwide will suffer a stroke during their lifetime". Many patients with stroke will be handicapped for the rest of their lives, and thus, managing risk factors and preventing stroke are important. Stroke is the fourth most common cause of mortality in Japan population. About 1,365,000

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patients have had a stroke and the death rate due to stroke is about 130,000 per year in Japan. In Japan, the mortality rate of stroke peaked between 1950 and the 1970s and decreased from the 1980s. It continues, however, to be the most common cause of incapacitation requiring nursing-care insurance.

Progress in Diagnostic Magnetic Resonance Imaging

Diffusion weighted (DWI) MR images are necessary for the diagnosis of acute ischemic stroke. Cell membrane damage increases intracellular water volume (cellular edema) that causes a decrease in the apparent diffusion coefficient (ADC) during acute ischemic stroke. Mismatches between DWI and perfusion weighted images (PWI) correlate with ischemic penumbra during acute early ischemic stroke on 3 Tesla MR angiograms and can diagnose occlusion or narrowing in the main trunk of cerebral arteries more effectively than conventional angiography. Such progress in imaging techniques has helped to effectively diagnose ischemic stroke during the acute phase.

Hemosiderin or deoxyhemoglobin accumulation in the brain is detected as black dots in T2*- gradient-recall echo (T2*-GRE) and susceptibility weighted (SWI) MR images, and they are described as microbleeds (MB) [1]. Such MBs in basal ganglia might correlate with atherosclerotic changes in small vessels whereas those in the cerebral cortex or sub-cortex are amyloid angiopathy. MB increase risk for intracranial hemorrhage, especially in the setting of anticoagulation. MB are also found in patients with ischemic stroke in deep brain lesions or lacunae infarction and they are associated with age, hypertension and diabetes [2, 3].

Intravenous Thrombolysis

A National Institute of Neurological Disorders and Stroke (NINDS) trial showed that treatment with intravenous (IV) alteplase (recombinant tissue-type plasminogen activator; tPA) administration within 3 hours of ischemic stroke onset improves clinical outcomes at 3 months, although it increases risk of symptomatic intracranial hemorrhage. Thus, IV t-PA therapy has changed the strategy for treating the super acute phase of ischemic stroke [4].

The downturn in the use of alteplase that occurred in October 2010 in Japan was

about 10 years behind that of the US and other countries. The dose of t-PA in Japan is 0.6 mg/kg, while a dose of 0.9 mg/kg is used in other countries because the risk of intracranial hemorrhage is higher among Japanese than Caucasians. The Japan Alteplase Clinical Trial (J-ACT) included 103 Japanese patients with 0.6% mg/kg alteplase administration. The outcomes and incidence of symptomatic intracranial hemorrhage were comparable to those published for 0.9 mg/kg. These findings indicate that 0.6 mg/kg of IV t-PA therapy might offer levels of clinical effectiveness and safety to Japanese patients that are compatible to 0.9 mg/kg in many other countries [5].

The European Cooperative Acute Stroke Study (ECASS 3) revealed the safety and efficacy of IV t-PA therapy within 3 to 4.5 hours of symptom onset. Although outcomes were better for alteplase than a placebo, the incidence of intracranial hemorrhage was higher with IV t-PA therapy. Serious adverse events and mortality rates did not significantly differ between the alteplase and placebo groups [6].

The benefit of intravenous thrombolysis for acute ischemic stroke decreases continuously over time from symptom onset [7], and each 15-minute reduction in the time to starting tPA treatment is associated with an increase in the odds of walking independently at discharge and being discharged to home rather than an institution. Furthermore, a 15-minute reduction in time to tPA treatment is associated with a decrease in the odds of death before discharge and symptomatic hemorrhagic transformation of infarction. Therefore, intravenous tPA treatment for acute ischemic stroke must be administered as soon as possible, rather than nearer the end of the time window [8].

Antiplatelet Therapy

Antiplatelet agents are used for both the primary and secondary prevention of ischemic stroke especially in individuals with risk factors for atherosclerosis. The International Stroke Trial (IST) and Chinese Acute Stroke (CAST) trials showed that aspirin administered during acute ischemic stroke led to a reduction of 11 nonfatal strokes or deaths per 1,000 patients in the first few weeks, but caused approximately two hemorrhagic strokes [9]. Thus, approximately nine non-fatal

CHAPTER 29

Impact of Dose Reduction on the Efficacy of Triple Therapy for Patients Infected with Genotype 1b and High Viral Loads

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Abstract: To examine the impact of dose reduction on the efficacy of pegylated interferon (PEG-IFN) plus ribavirin (RBV) plus telaprevir (TVR) triple therapy for patients infected with genotype 1b and high viral loads.

A total of 51 patients were recruited for this study. Patients were divided into groups receiving either 2,250 mg of TVR for 12 weeks and 600 – 1,200 mg of RBV for 24 weeks according to body weight (Group 1, *N* = 39) or 1,500 mg of TVR for 12 weeks and 400 mg of RBV for 24 weeks (Group 2, *N* = 13) plus 1.5 μg/Kg (range: 1.3 - 2.0 μg/Kg) of peg-IFN alpha-2b for 24 weeks. Patients of Group 1 were less than 65 years old or IL28 non T/T and over 65 to less than 70 years old. Patients of Group 2 were IL28B T/T and over 65 to less than 70 years old or over 70 years old.

Rapid virological response (RVR) rates were 66.7% in Group 1 and 84.2% in Group 2 (NS). Early virological response (RVR) rates were 79.5% in Group 1 and 91.7% in Group 2 (NS). End of treatment response (ETR) rates were 71.8% in Group 1 and 69.2% in Group 2 (NS). Sustained virological response (SVR) rates were 60.5% in Group 1 and 69.2% in Group 2 (NS). In multivariate analysis, significant contribution factors for SVR were IL28B (genotype TT; OR 1.83, P = 0.0032) and platelet counts (< 120,000; OR 2.14, P = 0.0140).

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It was concluded that the treatment strategy of dose reduction based on patient background (age and IL28B SNP polymorphism) was proper in Japanese patients.

Keywords: Dose reduction, Genotype 1b, HCV, Triple therapy, TVR.

INTRODUCTION

Hepatitis C virus (HCV) infection is estimated to affect 170 million individuals worldwide [1], including two million people in Japan. Chronic HCV infection often progresses into liver cirrhosis including the development of associated complications such as decompensated cirrhosis and hepatocellular carcinoma over the course of 20 to 50 years [2]. Successful treatment of HCV can result in viral eradication, which has been associated with a reduced incidence of hepatic decompensation and HCC in addition to prolonged survival. The recent development of triple combination therapy, consisting of pegylated interferon (PEG-IFN), ribavirin (RBV) and a protease inhibitor, telaprevir (TVR) or boceprevir (BOC), has improved the sustained virological response (SVR) rate of patients infected with genotype 1 and high viral loads [3, 4]. However, the side effects of these triple therapies may be too severe for patients with comorbid conditions such as anemia and depression. Furthermore, many patients develop skin rash and appetite loss, resulting in premature termination of treatment [3, 4].

In this study, we examined the efficacy of dose reduction of both RBV and TVR as compared to that of the standard dose treatment.

METHODS

Patients

A total of 51 patients were recruited for this study. All patients were infected with HCV genotype 1b and had a high viral load of more than 5.0 log IU/ml as determined by the HCV COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of this assay was 1.2 - 7.8 log IU/ml and undetectable samples were defined as negative. All eligible patients were required to satisfy the following criteria: (1) liver biopsy within three months of the start of therapy; (2) diagnosis of chronic active hepatitis by conventional classification; (3) positive

for HCV-RNA of genotype 1b in serum within three months in titers of > 5.0 log IU/ml by the HCV COBAS TaqMan HCV test; (4) abnormal serum alanine aminotransferase levels for more than six months; (5) leukocyte count > 3,000/mm^3, platelets > 100,000/mm^3; (6) serum bilirubin < 2.0 mg/dl; (7) lack of liver cirrhosis, hepatocellular carcinoma, autoimmune hepatitis, alcoholic liver disease and any other chronic liver diseases (positive for serological markers of hepatitis B virus); (8) lack of psychiatric illnesses, including depression, or conditions affecting the bone marrow, alimentary, cardiovascular, or pulmonary systems; and, (9) no immunosuppressive or anti-viral therapy within six months prior to entry, as described previously [5].

Treatment Protocol

Patients were treated with the combination therapy of peg-IFN alpha-2b plus ribavirin plus telaprevir: Median dose was 1.5 μg/Kg (range: 1.3 - 2.0 μg/Kg) of peg-IFN alpha-2b subcutaneously administered once a week; oral ribavirin was administered twice daily for a total dose of 400 – 1,200 mg; oral telaprevir was administrated once daily at 100 mg.

A total of 51 patients were recruited for this study. Patients were divided into groups receiving either 2,250 mg of TVR for 12 weeks and 600 - 1200mg of RBV for 24 weeks according to body weight (Standard dose protocol: Group 1, $N = 39$) or 1,500 mg of TVR for 12 weeks and 400 mg of RBV for 24 weeks (Reduced dose protocol: Group 2, $N = 13$) plus 1.5 μg/Kg (range: 1.3 - 2.0 μg/Kg) of peg-IFN alpha-2b for 24 weeks. Patients of Group 1 were less than 65 years old or IL28 non T/T and over 65 to less than 70 years old. Patients of Group 2 were IL28B T/T and over 65 to less than 70 years old or over 70 years old.

This study was approved by the Institutional Review Boards of participating clinical sites prior to study initiation, and the study was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all patients.

SNP Genotyping and Quality Control

Because the two reported significant *IL28B* SNPs (rs8099917 and rs12979860)

CHAPTER 30

Characteristics of Acute Cholangitis and Endoscopic Management of Elderly Patients at Our Institute

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Abstract: The clinical presentation of acute cholangitis, etiology and complications from endoscopic early biliary drainage in elderly patients was evaluated.

205 patients with acute cholangitis treated at the Kure Medical Center and Chugoku Cancer Center were enrolled. Of these, 108 patients were 75 years old or older (elderly) and 97 patients were younger than 75 years old. Patients' clinical characteristics, etiology and severity of cholangitis, and complications of endoscopic early biliary drainage were compared between the elderly and younger cohorts.

There was a significant difference in severe cholangitis between elderly and younger patients, at 17.6% and 0%, respectively. There was no significant difference in complications between elderly and younger patients, and between patients with and without early biliary drainage. One 91-year old woman with early biliary drainage had post-ERCP (endoscopic retrograde cholangiopancreatography) aspiration pneumonia and died.

Acute cholangitis in elderly patients may become severe in degree with a high incidence. Early ERCP for elderly persons is relatively safe, but it should be done carefully, not induce a consequent death due to complications such as aspiration pneumonia.

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Keywords: Acute cholangitis, Aspiration pneumonia, Biliary drainage, Complications, Elderly patients.

INTRODUCTION

There are several factors for determining the mortality of acute cholangitis. Old age is one of most important factors relating to a poor immune status, a poor nutritional condition, and an etiology of cholangitis. We retrospectively compared the clinical characteristics, prognosis, and safety of endoscopic biliary drainage between elderly and younger patients.

PATIENTS AND METHOD

From January 2009 to December 2011, 205 patients with acute cholangitis were treated at our institute. Of these, 108 patients were elderly at 75 years old or older (Group A) and 97 were younger than 75 years old (Group B). Patients with suspected cholangitis were included, while patients with acute cholangitis caused by neoplasm were excluded. Diagnosis of acute cholangitis and its severity were diagnosed using the guidelines for the *Management of Acute Cholangitis and Cholecystitis* (1st Edition, 2005, Japan). One item from category A (fever, abdominal pain or jaundice) and two items from category B (cholestasis, systemic inflammation or biliary abnormality) are necessary for the suspected diagnosis (Table **1**).

Table 1. Diagnosis of acute cholangitis (1st edition 2005, Japan).

A.	1. Fever
	2. Abdominal Pain
	3. Jaundice
B.	4. Elevated ALP and/or γ-GTP
	5. Elevated WBC and/or CRP
	6. Biliary dilatation, biliary stenosis, biliary stone
Definite diagnosis:	All of A or one item in A + All of B
Suspected diagnosis:	one item in A + two item in B

Table **2** shows the criteria for severity of cholangitis. One item was necessary to

diagnose severe cholangitis from following five items: shock, bacteremia, disturbance of consciousness, acute renal failure, and respiratory dysfunction. Because bacteremia was unknown on arrival of the patients to the hospital, we assessed severity, while excluding the item of bacteremia. One of the following items (jaundice, hypoalbuminemia, renal failure, reduced platelet count, and high fever) was necessary to diagnose moderate cholangitis. Urgent biliary drainage for severe cholangitis and early biliary drainage for moderate cholangitis were recommended as treatments for patients.

Table 2. Criteria for severity of acute cholangitis (1st edition 2005, Japan).

Severe (need one item)	
	① Shock
	② Bacteremia
	③ Disturbance of consciousness
	④ Acute renal failure
	⑤ Respiratory dysfunction
Moderate (need one item)	
	① Jaundice （T-bil ＞2.0mg/dL)
	② hypoalbuminemia（Albumine＜3.0g/dL)
	③ Renal failure（BUN>20mg/dl, Creatinine＞1.5mg/dL)
	④ Reduced platelet count（＜12×10⁴/mm2)
	⑤ High fever (>39℃)
Mild	Mild cholangitis does not meet the criteria of severe or moderate cholangitis

We evaluated patients' clinical characteristics, etiology and severity of cholangitis upon arrival and at 24h after admission, and for complications of emergent endoscopic retrograde cholangiopancreatography (ERCP) between Groups A and B. Statistical analysis was performed using the χ^2 test and Student's t-test. Differences were considered significant at $p < 0.05$.

RESULTS

Patient characteristics are shown in Table **3**. The mean ages of Groups A and B were 83 and 61.6 years old, respectively. In both groups, the most frequent etiology of cholangitis was common bile duct stones. A complicated history of heart disease and cerebral vascular disease was significantly higher in Group A than in Group B. There was no significant difference in the use of anti-platelet agents and/or anticoagulant agents.

CHAPTER 31

Hyperbaric Oxygen Therapy for Salvage of Flaps with Unstable Blood Flow

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Abstract: In plastic surgery, hyperbaric oxygen therapy is used to promote healing of intractable ulcers caused by severe diabetes mellitus, peripheral circulatory disorders, and severe scald burns. We performed hyperbaric oxygen therapy to improve blood flow disorders of transferred flaps and reported the utility of and problems with this method.

We performed hyperbaric oxygen therapy for 10 patients with postoperative blood flow disorder after flap transfer. All cases were reconstruction using pedicled flaps. Subject disorders were intractable ulcers with myelitis in three cases, decubitus ulcers in three cases, gas gangrene in one case, injury in one case, hair loss in one case, and defect after tumor resection in one case. Among the 10 patients for whom we performed hyperbaric oxygen therapy, eight experienced local chronic inflammation or systemic wound healing protraction factors.

The transferred flaps in 4 of 10 patients treated with hyperbaric oxygen therapy were salvaged, and the diseases were cured. In one patient, the flap was saved; however, myelitis symptoms were caused by an intramedullary bone screw. The other five patients showed epidermal or adiposal partial necrosis of the transferred flap and closed wounded area.

We performed hyperbaric oxygen therapy for 10 patients with unstable flap blood flow.

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A therapy effect was apparent in all cases, and supplemental surgical procedures were not required in five cases. Hyperbaric oxygen therapy is a useful flap salvation method for institutions that have access to hyperbaric oxygen devices.

Keywords: Flap salvage, Hyperbaric oxygen therapy, Negative pressure wound therapy, Pedicled flap, Skin graft.

INTRODUCTION

In hyperbaric oxygen therapy, patients inhale oxygen at high concentrations for planned pathologic amelioration. Hyperbaric oxygen therapy improves disease states through physical action, chemical action, or biological action by high-concentration oxygen inhalation in a high atmospheric pressure environment. Hyperbaric oxygen therapy is used to treat peripheral circulatory disorders such as Buerger's disease or carbon monoxide intoxication because the high atmospheric pressure environment drastically improves blood circulation [1 - 4]. This method is also used to treat necrotizing fasciitis caused by anaerobic bacteria using the chemical action of the hyperbaric oxygen therapy. For decompression sickness, the physical action of high-pressure force removes gases that become foam in the blood [5 - 8].

Skin grafts, as well as local, pedicled, and free flap transfers, are reconstruction methods for tissue defects caused by severe trauma and after large tumor resection. However, flap transfer is also required for defects with exposed cortical bone, important neurovascular tissue, and poor blood flow with chronic infectious disease. For these cases, some local flaps and pedicled skin flaps can experience partial necrosis by ischemia, congestion of blood circulation, and high rotation tension of the flap after flap surgery. We performed hyperbaric oxygen therapy to improve blood flow disorders of transferred flaps and reported the utility of and problems with this method.

MATERIALS AND METHODS

At the National Hospital Organization Kure Medical Center and Chugoku Cancer Center (Hiroshima, Japan) from 2010 to 2013, we performed hyperbaric oxygen therapy for 10 patients, including nine patients with postoperative blood flow

disorder after flap transfer and one patient with a deprivation injury. The therapy group comprised four men and six women ages 27 to 70 years (mean age, 60.0 years) at the time of initial diagnosis. All were reconstruction cases using pedicled flaps. We started hyperbaric oxygen therapy at a point when the blood flow disorder caused by ischemia or congestion was confirmed. Hyperbaric oxygen therapy was discontinued at flap survival. Subject disorders were intractable ulcers with myelitis in three cases, decubitus ulcers in three cases, gas gangrene in one case, injury in one case, hair loss in one case, and defect after tumor resection in one case. Among the 10 patients for whom we performed hyperbaric oxygen therapy, eight experienced local chronic inflammation or systemic wound healing protraction factors (Table **1**).

Table 1. Patient characteristics.

No.	Age (years)/sex	Primary diagnosis	Affected area	Delayed wound healing factor
1	27/F	Osteomyelitis	Femoral region	Chronic arterial obstruction, chronic inflammation
2	47/M	Gas gangrene	Lower limb	Diabetes mellitus
3	33/F	Injury	Lower limb	None
4	64/F	Osteomyelitis	Lower limb	Chronic inflammation
5	44/F	Decubitus ulcer	Buttocks	Spinal injury, chronic inflammation
6	76/F	Osteomyelitis	Lower limb	Diabetes mellitus, chronic inflammation
7	57/M	Hair loss	Head	None
8	60/M	Decubitus ulcer	Buttocks	Spinal injury
9	70/M	Decubitus ulcer	Trochanter	Spinal injury
10	56/F	Defect after tumor resection	Chest wall	Post chemotherapy

HYPERBARIC OXYGEN THERAPY DETAILS

We used an 8 m full-length teletherapeutic unit for all patients. Patients were gradually exposed to hyperbaric oxygen at two atmospheres absolute (approximately the same pressure as that at 10 m depth of water) for about 15

CHAPTER 32

Efficacy of Wound Closure with Cyanoacrylate Glue for Total Knee Arthroplasty without Drain

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Abstract: New surgical wound closure methods have been developed, and octyl-2-cyanoacrylate has been used for wound closure after surgery. In this study, we compared clinical outcomes for total knee arthroplasty with a new wound closure using cyanoacrylate glue without drain and conventional skin suture procedure with drain. Complications in the group of cyanoacrylate included one case of delayed skin healing. No case of blood transfusion, pulmonary embolism, deep-venous thrombosis, or deep infection existed. Concerning estimated blood loss, the group of cyanoacrylate decreased significantly at the time of post-op; 1 day and post-op, 3 days. This procedure had some benefits for patients and clinical staff.

Keywords: Blood loss, Cyanoacrylate glue, Drainage, Total knee arthroplasty, Wound closure.

INTRODUCTION

In recent years, new surgical wound closure methods have been developed. For example, octyle-2-cyanoacrylate (DERMABOND®; Ethicon, Somerville, New

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Jersey) was used in Japan from 2000 for low-tension wound closure. Data on the strength of incisions closed with Octyle-2-cyanoacrylate are limited [1].

However, Miller *et al.* reported that DERMABOND® is superior to staples in high-tension wound care [2].

On the one hand, drainage techniques have used after total knee arthroplasty because of its ability to decrease infection risk and increase range of motion [4].

In recent years, some authors have reported that drainage after total knee arthroplasty is not an effective clinical outcome compared to that of non-drainage [5, 6].

Therefore, we conducted this study to compare the efficacy of clinical outcomes for total knee arthroplasty with a wound closure using octyle-2-cyanoacrylate glue without drain and conventional skin closure procedure with drain.

PATIENTS AND METHODS

Between July 2010 and August 2012, all patients who were scheduled for a primary total knee arthroplasty at the Kure Medical Center, Hiroshima, Japan were included. Exclusion criteria included a history of septic arthritis, any hip or knee fracture, and rheumatoid arthritis. A total of 125 patients (140 knees) undergoing total knee arthroplasty for osteoarthritis knee were recruited. One surgeon performed all surgeries. The surgical procedures were as per the surgeon's routine practice. We did not use a pneumatic tourniquet around the upper part of the thigh. A midline skin incision and medial parapatellar capsular approach was made to expose the knee joint. Standard surgical techniques for intraoperative hemostasis were used. An appropriate type and size of knee prosthesis (Scorpio NRG [Stryker, Mahwah, New Jersey] or GenesisII [Smith&Nephew, Memphis, Tennessee]) was used. All components were cement-less prosthesis. No patellae were resurfaced.

Patients were randomized into 2 groups, (1) skin closure using DERMABOND® (Group D) and (2) skin closure using 4-0 nylon sutures (Group C). No surgical drains were used in Group D. Surgical drains were used with Group C, and we removed the drains for 2 days after surgery (Fig. **1**).

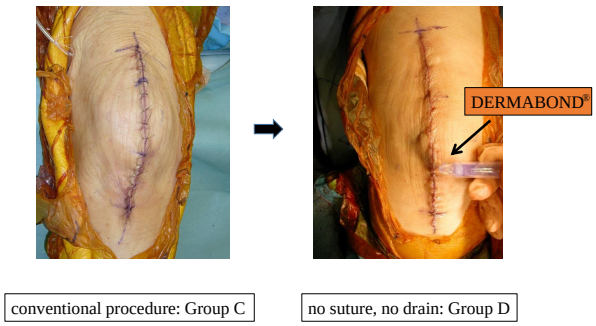

Fig. (1). Our surgical procedure for total knee arthroplasty. When we close the wound after implantation, we use DERMABOND® instead of skin suture, and we did not use suction drain.

Prophylaxis against venous thromboembolism was administered as per standard practice at our hospital with low molecular weight heparin for 7 days after surgery.

All patients had a standard post-operative program of analgesic medication and daily physiotherapy including the use of continuous passive motion machines.

Daily postoperative hemoglobin levels were measured at 1st and 3rd days after surgery.

The primary outcome was blood loss as calculated from the difference between preoperative and post-operative hemoglobin at 1st and 3rd days after surgery. Based on hemoglobin balance, the estimated blood loss was calculated according to the formula described by Good *et al.* [7] and Nadler *et al.* [8].

The secondary outcomes included the rate of perioperative blood transfusion, rate of surgical infections, length of hospital stay, and post-operative range of motion at 3rd day after surgery and discharge from our hospital. A student's *t*-test was used to evaluate and compare the patients in Group C and D *versus* the patients in group D. The *p*-value of <0.05 was considered significant.

RESULTS

Group C consisted of 61patients (68 knees: 9 males, 59 females), and Group D consisted of 64 patients (71 knees: 8 males, 63 females).

CHAPTER 33

Effects of Switching from Allopurinol to Febuxostat in Chronic Kidney Disease Patients

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Abstract: Febuxostat, a new xanthine oxidase (XO) inhibitor, could become the standard for managing uric acid levels in patients with chronic kidney disease (CKD). However, little has been reported regarding patients with severe renal impairment. Further, the conversion rate for switching patients from allopurinol to febuxostat remains unknown.

We studied 65 CKD patients being administered allopurinol for hyperuricemia and then switched them to febuxostat at a conversion ratio of 100 mg allopurinol: 10 mg febuxostat. Serum uric acid and creatinine levels were measured before and 4–8 weeks after the switch.

Sixty-three patients remained after excluding those who had discontinued treatment. There was no significant difference in serum uric acid and creatinine levels before and after the switch. Further, no significant differences were observed in serum uric acid levels before and after the switch when patients were stratified into diabetic and non-diabetic groups or when classified per gender. We divided patients into G1–G3b and G4–G5 groups depending on the stage of CKD; there were no significant differences in the G1–G3b group after the switch, but there was a significant decrease in serum uric acid levels in the G4–G5 group ($p < 0.05$).

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We demonstrated that 100 mg allopurinol and 10 mg febuxostat had equivalent hypouricemic effects in CKD patients and that these drugs did not affect serum creatinine levels. Thus, 10 mg febuxostat may have greater hypouricemic effects in patients with advanced CKD.

Keywords: Alopurinol, Chronic kidney disease, Febuxostat, Uric acid, Xanthine oxidase.

INTRODUCTION

Chronic kidney disease (CKD) is a concept that was first proposed by the National Kidney Foundation in the U.S. in 2002. An estimated 10% of the adult Japanese population suffers from CKD [1], making CKD a common disease. Hyperuricemia is frequently observed in CKD patients and is also a risk factor for CKD progression; therefore, it is one of the therapeutic targets of CKD therapy [2].

Antihyperuricemic agents are broadly divided into uricosuric agents and uric acid synthesis inhibitors. Benzbromarone and probenecid are uricosuric agents that act on the renal tubules to prevent the reuptake of uric acid, but their effects are weakened in CKD patients with reduced GFR; moreover, their use is associated with the risk of renal tubule occlusion by uric acid calculi. Allopurinol, a uric acid synthesis inhibitor, is a xanthine oxidase (XO) inhibitor developed during the development of anticancer drugs after it was observed to decrease uric acid levels. In 1988, George Hitchings and Gertrude Elion were awarded the Nobel Prize in medicine for the development of allopurinol [3]. Traditionally, allopurinol, among all other uric acid synthesis inhibitors, was commonly used to treat hyperuricemia in CKD patients. However, it is known to cause hepatic dysfunction, vasculitis, and dermatitis, and it also occasionally causes severe renal failure [4, 5]. Allopurinol hypersensitivity syndrome is observed in 0.4% patients who receive allopurinol and can lead to Stevens–Johnson syndrome, a serious disorder of the skin and mucous membranes. This condition may sometimes be life-threatening [6]. The metabolically active form of allopurinol, oxypurinol, is excreted *via* the kidneys, and therefore, CKD patients have elevated serum oxypurinol concentrations. For this reason, there is a high rate of adverse reactions to

allopurinol in patients with advanced CKD, which makes the management of uric acid levels in CKD patients difficult.

Febuxostat is a new XO inhibitor that was first used clinically in Japan in 2011. Febuxostat does not have the purine skeleton that is the underlying cause of adverse reactions to allopurinol. Further, it is excreted not only *via* the kidneys but also *via* the liver following glucuronidation [7]. Its use in CKD patients is expected to result in a lower onset frequency of adverse reactions, and it has the potential of becoming the standard antihyperuricemic agent for CKD patients. However, febuxostat has rarely been used in patients who have severe renal impairments and the rate of switching patients from allopurinol to febuxostat remains unknown.

Thus, in this study, we switched hyperuricemic patients with CKD who were receiving allopurinol to febuxostat and investigated the outcomes and the incidence of side effects.

METHODS

The subjects included 65 hyperuricemic CKD patients being treated with allopurinol at the National Hospital Organization Kure Medical Center. We used a conversion ratio of 100 mg allopurinol:10 mg febuxostat. Subjects were not switched to any drug other than febuxostat. Serum uric acid and creatinine levels were measured before and 4–8 weeks after the switch. Subjects who experienced attacks of gout, worsening of subjective symptoms that were considered to be adverse reactions, or adverse events that manifested as abnormalities in blood test data were immediately withdrawn from the study. All results are shown as the mean ±standard deviation. Data were analyzed by the Wilcoxon signed rank test. $P < 0.05$ was considered significant in all analyses.

RESULTS

Of the 65 CKD patients who were switched from allopurinol to febuxostat at a conversion ratio of 100 mg:10 mg, febuxostat administration was discontinued in two patients due to adverse events. The backgrounds of the remaining 63 patients (45 men and 18 women, mean age = 65.1 ± 14.3 years) are shown in Table **1**. Of

CHAPTER 34

Infection Control Program for MRSA in Intensive Care Units

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Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is causing increased morbidity and mortality in intensive care units (ICUs). Its presence causes several challenges in implementing infection control measures. This study determined the incidence of MRSA acquisition in the ICUs and evaluated interventions to reduce the rate of MRSA acquisition.

A prospective study was conducted from April 2004 to March 2013 in the ICUs of our hospital that is a district teaching hospital in Japan. Patients were screened for MRSA with their sputa, nose or throat swabs on admission. MRSA acquisition was defined when negative on admission screening and positive after admission in hospital. The MRSA control program consisted of four practices: 1) frequent oral care with brushing from April 2006, 2) the use of a closed suction system for all patients receiving mechanical ventilation from December 2006, 3) reinforcement of standard precautions for patient contact, with emphasis on hand hygiene, by the supervisory nurse from August 2007, and 4) cleaning equipment and environment with alcohol swab from September 2007.

Of the 9,401 patients examined, 570 (6.06%; 2.9%-9.65%/year) had MRSA on admission. MRSA acquisition rates in the ICU per 1,000 MRSA-negative patients

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decreased significantly from 28.56 in the three years before the interventions to 14.01 in the 6 years after the interventions ($p<0.05$).

Although MRSA infection in ICUs has continued, we succeeded in decreasing MRSA acquisition in the ICUs by routine long-term MRSA screening on admission and a MRSA control program.

Keywords: Acquisition, ICU, Infection control, Intervention, MRSA.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) infection has caused increased morbidity and mortality, and infection control measures have become difficult in intensive care units (ICUs). The incidence of MRSA acquisition in the ICUs was examined and the effect of interventions was evaluated to determine whether they reduced the rate of MRSA acquisition or not in this study.

METHODS

Setting

The National Health Organization Kure Medical Center and Chugoku Cancer Center in Japan is a 700-bed district teaching hospital. The medical-surgical ICUs have 17-beds with 8 isolation rooms without negative air pressure for trauma and critically-ill patients, with approximately 1,400 patients admitted annually. In 2013, the average hospital stay was 6.1 days in length.

Study Design

This study was conducted prospectively from April 2004 to March 2013. Patients admitted to the ICUs during this period were enrolled in this study. MRSA screening for surveillance cultures within 24 hours after admission were done with their sputa, nose or throat swabs samples obtained. During ICU stay, clinical samples were also collected for microbiological analysis.

The basic infection control policy enforced by the Infection Control Team of our hospital requires: (1) strict isolation of a MRSA-positive patient as soon as possible, (2) rules for hand hygiene, and (3) routine glove and apron use for each

patient contact.

A carrier who acquired MRSA prior to ICUs admission was defined as a patient with MRSA growth from admission screenings. MRSA acquisition was defined as negative on admission screening and positive with respiratory samples 72 hours or longer after admission.

MRSA Control Pogram

The MRSA control program consisted of four phases: 1) frequent oral care with brushing at least every 8 hours from April 2006, 2) daily use of a closed suction system for all patients receiving mechanical ventilation from December 2006, 3) reinforcement of standard precautions for patient contact emphasizing hand hygiene with alcohol hand rub gel by the supervisory nurse from August 2007, and 4) cleaning equipment and environment with alcohol swab from September 2007.

The rate of MRSA colonized patients on admission was calculated as cases per 1,000 admissions. The rate of ICU acquisition was estimated as the number acquiring MRSA per 1,000 MRSA-negative screening admissions. We compared the data from the initial four years with data from the last six years using a $\chi 2$-test with Ryan's method. A *p*-value of less than 0.05 was considered significant.

RESULTS

A total 9,401 patients admitted to ICUs were enrolled in this study. Of these patients, 570 (6.06%; 2.9%-9.65%/year) had MRSA on admission to the ICUs (Table **1**).

Table 1. Carriers of MRSA on admission to Intensive care unit.

Period Year/Month	Number of patients admitted	Number (%) of MRSA-positive patients	Number of MRSA-negative patients	MRSA carrier rates per 1000 patients admitted
2004/4 - 2005/3	815	60 (7.36%)	755	73.62
2005/4 - 2006/3	902	87 (9.65%)	815	96.45
2006/4 - 2007/3	1276	80 (6.27%)	1196	62.69
2007/4 - 2008/3	1185	80 (6.75%)	1105	76.51
2008/4 - 2009/3	1369	74 (4.89%)	1295	54.05
2009/4 - 2010/3	1092	72(6.59%)	1020	65.93
2010/4 - 2011/3	982	61(6.21%)	921	62.12
2011/4 - 2012/3	848	29(3.42%)	819	34.2
2012/4 - 2013/3	932	27(2.9%)	905	28.97

CHAPTER 35

Prevention and Management of Persistent Postoperative Pain – A Review of Literature and A Proposal of Therapeutic Strategy

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Abstract: Recent studies have indicated a high prevalence of persistent postoperative pain (PPP) in patients who undergo various surgical procedures. Patient suffering from PPP and the cost to manage this pain are substantial socioeconomic burdens. Although recent studies have revealed various underlying mechanisms of PPP, prevention and management remains unsatisfactory. In this review, we briefly summarize the current understanding of etiology and pathophysiology, which include neuropathic, nociceptive, inflammatory, and myofascial pains as well as psychological or psychiatric factors, and advances in the prevention and management of PPP over the last decade. In addition to the literature review, we present findings from our observation studies of patients with post-thoracotomy pain syndrome, which indicate myofascial pain as a major pathophysiological cause of PPP. Based on the literature review and our recent clinical studies, we propose a comprehensive practical strategy for the prevention and management of PPP.

Keywords: Myofascial pain, Neuropathic pain, Persistent postoperative pain, Post-thoracotomy pain syndrome, Sensitization of nociceptive neurons.

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INTRODUCTION

In 1998, Crombie *et al.* [1] reported the results of a cohort study on the etiology of chronic pain among 5,130 patients who consulted 10 outpatient pain clinics in the UK. They found that approximately 40% of the patients suffered from chronic pain after surgery or trauma. Surgery contributed to pain in 22.5% of patients. Accordingly, the researchers emphasized the importance of prevention and management of persistent postoperative pain (PPP). Recent studies have indicated a high prevalence of PPP in patients who have undergone various surgical procedures [2 - 6]. Actually, patients' suffering from PPP and the cost to manage this pain are substantial socioeconomic burdens [3, 4]. Although recent studies have revealed various underlying mechanisms of PPP, prevention and management remains unsatisfactory [2 - 4]. In this review, we briefly summarize the current understanding of etiology, pathophysiology, and advances in the prevention and management of PPP over the last decade. In addition to the literature review, we present findings from our observation studies of patients with post-thoracotomy pain syndrome (PTPS) and propose a practical strategy for the management of PPP.

I. DIAGNOSIS AND PREVALENCE

A. Definition, Differential Diagnosis, and Incidence of PPP

PPP is defined as postoperative pain that persists more than 3 months after a surgical procedure [3]. Although pain lasting at least 2 months after a surgical procedure is used by some authors [4, 7], 3 months seems more clinically relevant as a definition to report epidemiological and clinical trials [3]. To diagnose PPP, we need to discriminate PPP from other pain that occurs after surgery. The differential diagnoses of PPP include the recurrence of tumor, infection, and other pathological causes other than surgical tissue injury [3].

B. Prevalence

The incidence of PPP has been reported from 5% to 80%, depending on the type

of surgery [2 - 6]. Additionally, 10% of patients with PPP have had pain severe enough to disturb quality of life [4 - 6]. A recent systematic review reported the median prevalence of PPP was 30-35% in thoracic surgery, 20% in surgery for bones and arthroplasty, and 10%-14% in abdominal surgery [6].

II. CURRENT UNDERSTANDINGS AND THERAPIES

A. Etiology

Many factors are assumed to cause PPP [3 - 6, 8 - 16] including age, preoperative physical and psychological conditions, type of surgery, anesthesia, and pre and postoperative pain management [3 - 6, 8 - 16]. Perioperative patient conditions, such as depression, insomnia, pain catastrophizing, and social stress, are also considered factors in the development of PPP [3 - 6, 8, 15]. Dysfunction of the intrinsic descending inhibitory system may also tend to result in PPP [10, 11]. Further, a state of hypervigilance may also contribute to the development of PPP [16]. Recent investigations have revealed that genetic factors play an important role in the increased sensibility to pain [3, 8]. Such genetic factor may underlie in certain patients with PPP [3, 8].

B. Pathophysiology

1) Neuropathic Pain

Neuropathic pain has been attributed as the major cause of PPP as evidenced by clinical findings of nerve injuries in the surgical wound area [3 - 6, 8]; prevalence of neuropathic pain differs by type of surgery. A recent systematic review reported the prevalence of PPP as 66% in thoracic surgery, 67.7% in mastectomy, 30.5% in repair of inguinal hernia, 33% in gynecological surgery, and 5.7% in total knee/hip arthroplasty [6]. Additionally, a high incidence of injury of intercostal nerves and their peripheral branches may elucidate higher prevalence of PPP in patients who undergo thoracic surgery [3 - 6]. Other factors that may cause neuropathic pain include the use of artificial prosthesis, such as mesh plugs in the repair of inguinal hernia; repetitive surgery; and chemotherapy and

CHAPTER 36

Bland-Altman Analysis for Method Comparisons

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Abstract: To assess the adoptability of a new measuring method when true values are unknown, it is necessary to compare the proposed with the conventional method. Although Bland-Altman correlation analysis is widely accepted, it is occasionally misused or applied where it is inappropriate. We provide an overview of Bland-Altman analysis and teach its application and the interpretation of its results. We also comment briefly on regression analysis, another comparison method.

Keywords: Bland-Altman analysis, Limit of agreement, Method comparison, Pitfall, Statistics.

BASIC CONCEPT OF BLAND-ALTMAN ANALYSIS

Before applying a new measuring method, it is necessary to assess the agreement between the new and the conventional method; this is known as the "reference standard". We provide an overview of Bland-Altman analysis, a comparison method that was first described by John M. Bland and Douglas G.Altman in the 1980s [1, 2]. Although criticized by some [3, 4], it is now widely accepted as the method to compare two different measuring techniques.

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In Bland-Altman analysis, the average of the results obtained with the new method and the reference standard is plotted on the horizontal (X) axis. The difference between the values obtained with the new method and the reference standard is plotted on the vertical (Y) axis. Lastly, the tendency of the resulting scattergram is visually assessed.

INTERPRETATION OF BLAND-ALTMAN ANALYSIS

Scattering of the plotted data above or below the zero line of the Y axis indicates a fixed bias and a tendency of the new method to show larger (above the line) or smaller (below the line) values than the reference standard. If there is a fixed bias, the new method is not interchangeable with the reference standard and a correlation factor is required to convert the results to the reference standard. If points on the scattergram tend to move away from the zero line of the Y axis as the value on the X axis increases, there is a proportional bias and the results acquired with the new method tend to differ from the reference standard as their value increases. If there is a proportional bias the new method cannot be the substitute for the reference standard. These two biases are known as the systemic bias.

The graph derived by Bland-Altman analysis is known as the "Bland-Altman plot". It facilitates visual recognition of the existence of systemic bias. Patterns of Bland-Altman plots are shown in Figs. (**1** to **4**). Needless to say, the null hypothesis of systemic bias between the new method and the reference standard must be rejected statistically.

When the 95% confidence interval of the difference between the new method and the reference standard includes zero, the data will scatter to both the positive and negative side of the zero line of the Y axis, indicating that there is no fixed bias. The 95% confidence interval of the difference is defined by the formula

$$d - t \times \sqrt{S^2/n}$$
$$d + t \times \sqrt{S^2/n}$$

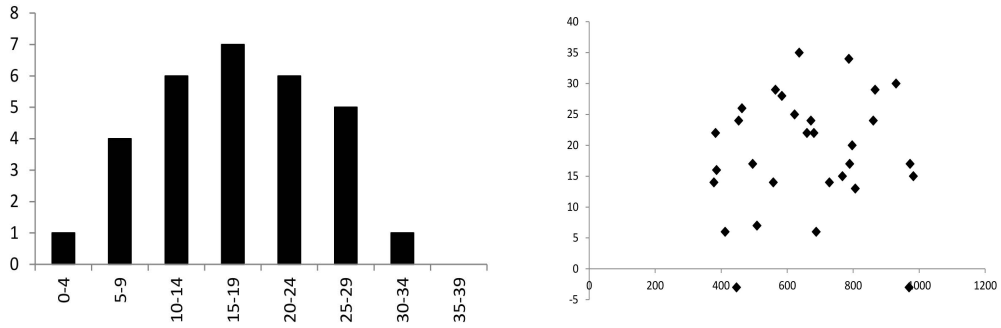

Fig. (1). (**A**) This histogram shows that the differences are normally distributed.
(**B**) On this Bland-Altman plot most data points are on both the positive and the negative side of the zero line of the difference. There is no tendency for an increase in the magnitude of the difference as the measurement increases. This indicates that there is no systematic bias. Adding the limit of agreement (LOA) line on the graph is optional.

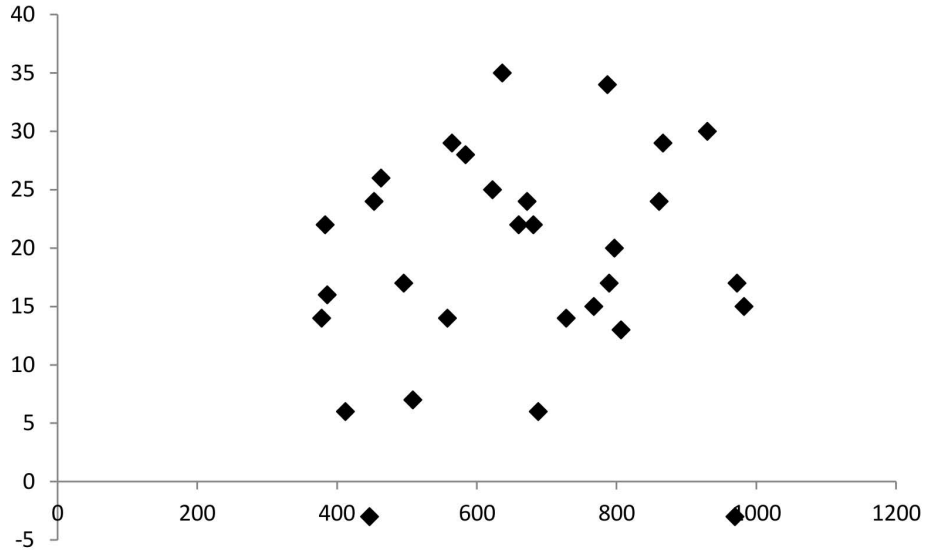

Fig. (2). On this Bland-Altman plot most of the data points are on the positive side of the vertical (Y) axis indicating there is a fixed bias.

CHAPTER 37

Prospective Study of the Treatment of Biotin in Patients with Drug Erythema due to Gefitinib or Erlotinib

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Abstract: Gefitinib and erlotinib, which are epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), have been used for the treatment of inoperable and recurrent non-small cell lung cancer patients. A major side effect of these drugs is skin rash, which occurs at a high frequency. Biotin is a water-soluble vitamin that belongs to the vitamin B family; biotin deficiency increases the risk of skin dermatitis. We recently reported that biotin was administered to four patients with skin rash, all of whom were treated with either gefitinib or erlotinib and were unable to be treated by a steroid ointment alone. Biotin may be considered useful in the treatment of skin rash caused by EGFR-TKIs. The aim of this paper is to discuss the usefulness of biotin in patients with erythema because of gefitinib and erlotinib.

Keywords: Biotin, EGFR-TKI, Erlotinib, Erythema, Gefitinib.

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IINTRODUCTION

Regarding the serious side effect of epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), acute pulmonary obstacle and interstitial pneumonia are well known [1]. However, according to the side effects, the effects of cutaneous symptoms have been reported frequently [1]. A major side effect of these drugs is skin rash, which occurs at a high frequency. The Asian international joint third aspect clinical trial (IPASS) of Gefitinib found 302 cases of skin rash and 66 cases of acne (60.7%), 102 cases of itching (16.8%), and 143 cases of skin drying (23.6%) in Asian patients [2]. Moreover, the domestic first aspect continuation examination and domestic second aspect clinical trial for the non-small cell lung cancer (NSCLC) (after second treatment) of erlotinib, reported 221 cases on rash (97.8%), 161 cases of skin drying (71.2%), 143 cases of itching (63.3%) in Japanese patients [3].

It is well known that biotin deficiency increases the risk associated with skin dermatitis [1]. We recently reported that biotin was administered to four patients with skin rash, all of whom were treated with either gefitinib or erlotinib and were unable to be treated by a steroid ointment alone [1]. In the patients who received EGFR-TKI, it is well known that it is difficult to control skin symptoms, even if a steroid ointment is applied.

METHOD

Biotin is a water-soluble vitamin belonging to the vitamin B family [1]. In a previous study, 6 mg/day of biotin was given three times a day until the progression of the cutaneous and skin symptoms were observed. According to the symptoms of the hands and feet, three points (exanthema, skin drying, skin reaction of hands and feet) were evaluated by Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. This prospective study was approved by the Ethical Review Board (2011/3/12 Kure Medical Center, Chugoku Cancer Center Ethical Review Board. Approval number 21-62).

DISCUSSION

According to the mechanism of dermatopathy due to the EGFR-TKI, the

inhibition of EGFR decreases the expression of Mitogen-activated protein kinase (MARK) and Ki-67, and increases p27kipl and phospho-STAT3, which facilitates the differentiation of keratinocyte in the epidermis basal layers and causes dyskeratosis [4]. Also, the inhibition of EGFR derives chemotactic factors and induces inflammation and apoptosis because of the neutrophils and lymphocytes, which facilitates thinning of the corner layers, increases apoptotic cells in the skin, forms plugging in the infundibular hair follicles, and causing acneiform exanthema [5].

It is well known that mild-deficiency Biotin induces dry skin with skin reddening and lymphocytic infiltration of skin and erosion partially [1]. In severe Biotin deficiency cases, severe erosion around the eyes, mouth, and anus are detected [6]. Biotin affects carboxyl transferase and acts as a coenzyme of enzymes such as acetyl CoA carboxylase, propionyl CoA carboxylase, and pyruvate carboxylase. However, the mechanism for ameliorating cutaneous symptoms remains unclear. We recently reported that biotin was administered to four patients with skin rash, all of whom were treated with either gefitinib or erlotinib and were unable to be treated by a steroid ointment alone [1]. Further basic and clinical trials may be needed to confirm the value of biotin treatment in patients who receive EGFR-TKI.

CONCLUSION

In this article, the authors discussed that biotin may be considered useful for the treatment of cutaneous symptoms caused by EGFR-TKIs. Further trials may be needed to confirm the value of biotin in this setting.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare for this publication.

ACKNOWLEDGEMENTS

Declared none.

CHAPTER 38

Talking about Life Expectancy with Our Cancer Patients Considering Palliative Chemotherapy

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Abstract: When making decisions about palliative chemotherapy, patients require information on how the cancer and its treatment will affect their life expectancy. To properly communicate this information, truthful and honest conversations about a patients' prognosis and the goals of treatment are essential, because any misunderstanding about the intent of treatment may lead to requests for ineffective or futile treatment. However, these conversations are inherently difficult due to a low confidence in an oncologist's ability to prognosticate accurately and fear of destroying hope or provoking emotional distress. There are few studies or guidelines for oncologists in estimating and explaining life expectancy in a way that conveys meaning without destroying hope. A recent study has demonstrated that providing estimates for worst-case, typical and best-case scenarios for survival are a helpful way of communicating life expectancy while conveying hope, and that the percentiles from an overall survival curve in first-line chemotherapy trials for advanced non-small cell lung cancer (NSCLC) can provide accurate estimates of the three scenarios. Furthermore, the initiation of establishment of illness understanding and discussing patients' goals for treatments of metastatic NSCLC earlier in the course provides a more accurate assessment of their prognosis, depression and survival. Additionally, decision aids (DAs) in the setting of advanced colorectal cancer and palliative chemotherapy has shown that the use of DAs improved patient understanding of prognosis, treatment options, risks, and benefits without increasing anxiety. This review explores communication issues when talking about life expectancy with our cancer patients participating in palliative chemotherapy.

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Keywords: Conversation, Life expectancy, Non-small cell lung cancer, Palliative chemotherapy, Prognosis.

INTRODUCTION

Patients with advanced cancer are often motivated to have chemotherapy near the end of life. In a study of Medicare patients who died with advanced cancer in the *Surveillance, Epidemiology, and End Results* dataset, rates of chemotherapy within 14 days of death increased between 1993 and 2000 [1]. Cancer patients who have poor illness understanding and overestimate their life expectancy are more likely to choose aggressive medical care at the end of life [2, 3]. An accurate understanding of treatment benefits and harm may lead to patients making more informed decisions that are aligned with their overall goals [4, 5]. Central to all of these goals is the need for realistic conversations throughout of the course of the disease. More recent studies have shown that discussing the effects of cancer treatments and establishing illness understanding early in the course of the disease decreases chemotherapy use near the end of life [6 - 8]. However, initiating conversations about a poor prognosis or supportive care alone after cancer progression is inherently difficult and can seem like giving up or admitting failure to patients [9]. The difficulty of these conversations may be due to low confidence by oncologists in their ability to prognosticate accurately and fear of destroying hope or provoking emotional distress [10]. Moreover, there is little guidance available for oncologists in estimating or discussing survival time for patients with advanced cancer at the early phase of their illness. In this review, we explore some of the implications that arise from talking about life expectancy with cancer patients participating in palliative chemotherapy.

ASK THE PATIENTS WHAT THEY WANT TO KNOW ABOUT PROGNOSIS

Most cancer patients want to know their life expectancy and specific estimates of best case, worst case, and typical case for survival [11]. However, not all patients want prognostic information, and not all who want it will request it. One study showed that only 70% and 60% wanted to know the likelihood of cure and their life expectancy, respectively [12]. Other patients might have psychosocial distress

that needs to be addressed prior to confrontation with prognosis [13]. Patient desires for information should be considered by first asking patients what they wish to know about prognosis, telling them desired information, and then asking, "What do you understand about your situation?" This is a paradigm known as the "Ask-Tell-Ask" approach [14, 15]. Moreover, a patient's preference for these disclosures can be dynamic and change with clinical situation [16]. Oncologists should repeatedly ask if patients want information about life expectancy, and if so, what type of information they want throughout the course of the illness.

DEFINE THE FOUR GOALS OF TREATMENT

Consideration of chemotherapy, whether curative or palliative, should be done in the setting of meeting the patient's goals of care [17]. If cure is not a realistic goal, the oncologist should tell the patients that the goal of therapy is not curative and is for the patient to do as well as possible for as long as possible. In order to break this concept down into more comprehensible pieces, it is separated into four related goals. These are that the oncologist would like the patient to have: the fewest side effects as possible from the cancer, the fewest side effects as possible from the treatment, the best quality of life, and the longest life [18]. These simpler concepts are useful when it is time to initiate or stop palliative chemotherapy and switch to alternative therapy.

GIVE TRUTHFUL AND HONEST INFORMATION

Oncologists need to give patients honest and truthful information about their life expectancy and treatment options. This is associated with more realistic prognostic estimates and decisions that are better aligned with patient wishes [19 - 22]. However, such discussion is difficult and many oncologists will avoid such conversations, be overly optimistic, or delay discussion until patients are close to death [23]. In a study of discussion between oncologists and incurable cancer patients, although most patients (75%) were informed of their incurability disease, only 58% of patients were told about life expectancy and fewer than 10% were given a time frame of life expectancy [24]. One of the main reasons for this reluctance to truthfully discuss life expectancy includes fear of reducing patient hope and causing distress [25]. In an advanced cancer population, patients who

CHAPTER 39

End-of-Life Care

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Abstract: We describe the significance of the end-of-life care for cancer patients based on our experience. During the terminal phase of cancer, particularly within the final two months, physical function rapidly declines and various symptoms appear; therefore, a prompt response is required to alleviate symptoms. Treatment must be selected in accordance with the patient's wishes to reduce their symptoms. Advance care planning (ACP) is primarily done by the patient and their family, and is sustained collaboratively by the supporting medical team, and not the unilateral "elicitation of a commitment" from the patient by the medical staff. ACP increases patient and family satisfaction during the end-of-life stage, and alleviates anxiety and depression among survivors after the death of a patient. Performing symptom control during the end-of-life stage of the patient is important with no demand for explanations. The use of morphine is recommended as drug therapy for dyspnea. Treatment of death rattle involves administering anticholinergic drugs and reducing intravenous (IV) infusions. It is important to provide families with explanations and make considerations for their inquiries. Routine assessments and corrections must be conducted using a guideline for intravenous infusions of 1,000 ml or less per day. Unbearable pain can remain in the end-of-life stage, even after palliative treatment has been performed. In these cases, sedation is performed after sufficient assessment of pain and after obtaining agreement from the medical team and consent from the patient and family following an explanation.

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Keywords: Advance care planning, End-of-life care, Sedation, Survival prediction, Symptom control.

INTRODUCTION

End-of-life is not clearly defined, but has been articulated as "the stage at which there is no chance of recovery through treatment and death is expected within about six months (http://www.ask.com/wiki/End-of-life care)". This period may differ by definition from three to twelve months. (http://www.ncpc.org.uk/sites/default/files/AandE.pdf). The course and end-of-life stage that patients go thru also varies according to their diseases.

During the terminal phase of cancer, particularly within the final two months, physical function rapidly declines and various symptoms appear; therefore, a prompt response is required to alleviate symptoms [1]. Treatment must be selected in accordance with the patient's desire to reduce their symptoms. Additionally, sufficient discussion is necessary to hear the patient's wishes and input from the family. As such, it is necessary to refrain from aggressive treatment and consider the ethical issues including sedation.

It is also necessary to respond to the patient and family anxieties. The patient and their family may need the physician to explain changes in symptoms during this time, and the attending physician has to be prepared to provide the proper responses. We always relieve the anxieties amongst patients and their families by explaining that we will strive to allow patients to go thru this period without pain by alleviating symptoms. These explanations occur during patient interviews or examinations.

Our facility and service handles one-hundred or more cancer patients each year, with palliative care doctors and other individuals in charge of palliative care teams. In this article, we describe the significance in the end-of-life care for cancer patients based on our experiences.

DISCUSSING TREATMENT AND CARE GOALS WITH PATIENTS AND FAMILIES

At the end-of-life stage, it is important for attending physicians to discuss further

treatment and care with their patients. Few reports have examined the intentions of patients, although a Ministry of Health, Labor, and Welfare study group released a report on this subject [2]. The reported noted that 71% of patients wish to quit life-prolonging treatment once their disease becomes terminal with no possibility of recovery (≤ 6 months to live), and 83.7% of medical staff are in favor of "respecting the patients intentions" through living wills. However, at present, only 14% of individuals have actually discussed living wills with surrogate decision makers or medical staff in Japan [3].

Discussing these goals is a collaborative interaction by the patients, their families, and the medical staff based on a patient's prior declarations of their intentions (advance directives) including DNAR (Do Not Attempt Resuscitation) orders (Fig. **1**) [4]. Advance care planning (ACP) is performed mainly by the patient and their family, and is sustained collaboratively by the supporting medical team, and should not be the unilateral "elicitation of a commitment" from the patient by the medical staff. ACP increases patient and family satisfaction during the end-of-life stage, and it alleviates the anxiety and depression of survivors after the death of a patient [5].

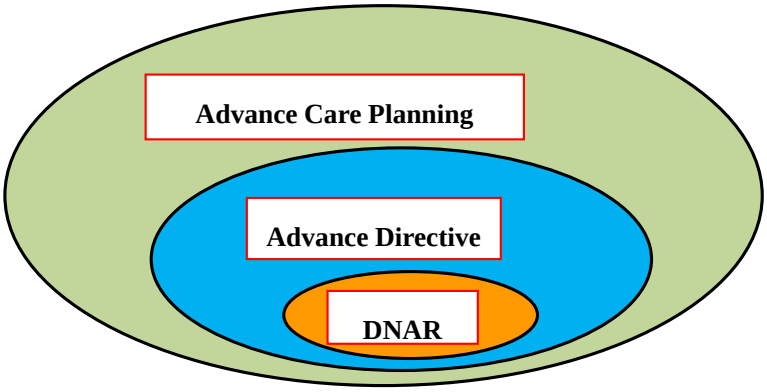

Fig. (1). Advance care planning [4].

The attending physician should aim to establish goals with patients and their families during these discussions based on shared information regarding a patient's conditions. These goals are established for the future to maintain hope and may vary and change according to the course of the disease. Thus, the role of medical staff is to support these goals in accordance with each patient's situation.

CHAPTER 40

Humanized Mouse Models as An Experimental Tool to Investigate Disease Immunology

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Abstract: Many studies of human immunology have been limited to *ex vivo* analysis due to ethical considerations. Detailed study, however, requires *in vivo* analysis, as *ex vivo* analysis of human immune cells does not always represent *in vivo* status. Therefore, small animal models have been used to overcome this limitation and several humanized mouse models that reproduce the human immune system have been developed. This review summarizes humanized mouse model characteristics and progress in their use for research of human diseases immunology.

Keywords: Humanized mouse model, Immunodeficient mouse, Immunology, Pre-clinical research, Translational research.

INTRODUCTION

"Humanized mice" are defined as immunodeficient mice that have been engrafted with human cells or tissues. This review focuses especially on humanized mice that have been engrafted with human haematopoietic cells and tissues that generate a functional human immune system in mice in order to investigate human immune function. The models are created by reconstituting a human

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immune system in immunodeficient mice that lack a main immune system component (T, B cells, and *etc.*), *e.g.*, SCID (severe combined immunodeficiency) mutation or derivatives including NOD/SCID mice, with human hematopoietic cells.

Many humanized mouse models have been developed thus far and are categorized into four main types depending on different experimental purposes. These include the Hu-PBL-SCID (Human Peripheral Blood Lymphocyte) mouse model, Hu-HSC-SCID (Human Hematopoietic Stem Cell) mouse model, SCID-Hu mouse model, and NOD/SCID-hu BLT (Bone marrow, Liver, Thymus) mouse model.

HUMANIZED MOUSE MODELS

Hu-PBL-SCID Mouse Model

Hu-PBL-SCID mice are immunodeficient mice transferred and engrafted with human peripheral blood mononuclear cells (PBMC) [1]. This model shows successful engraftment of PBMC and the secondary immune response of donor human individuals. This model is useful for studying allograft rejection, evaluation of human T cell specific drugs *in vivo* [2], and human specific infectious pathogens [3]. This model, however, has several limitations, including low levels of B cells or myeloid cells engraftment, difficulty in reproducing primary immune responses [4, 5] and activation of transferred T cells, perhaps owing to homeostatic proliferation [6], and xenoreactivity against host mouse antigen [7], resulting in T cell anergy and lethal xenograft-*versus*-host disease (xenoGVHD).

Hu-HSC-SCID

Mouse Model Hu-HSC-SCID mice are immunodeficient mice engrafted with human hematopoietic stem cells (HSC) [8]. In this mouse, HSC engraftment results in a repopulation of B cells as well as T cells. The development of hematopoietic cell from HSC in such mice should prevent homeostatic proliferation and lethal xenoGVHD through a negative selection mechanism by MHC antigens of the host mouse. In the initial period, the human HSCs engraftment in this model was insufficient and frequently lacked human T cell

development [9], although diverse immunoglobulin repertoires of B cells were observed [10]. However, recent progress in the development of immunodeficient mouse strains with a mutation in the interleukin-2 (IL-2) receptor γ-chain locus (Il2rγ; also known as γc and CD132) has led to more effective development of a human immune system in this model (described in detail below). These immunodeficient recipients have shown the development of a naïve human immune system, generation of thymopoiesis, and multi-lineages of hematopoietic cells including T, B, NK and antigen presenting cells (APC), T cell dependent [11] or independent antibody production [12], and intrathymic development of regulatory T cells [13 - 15]. Furthermore, T cell development in the periphery was observed after T cell depletion [16], indicating that this model should be useful pre-clinically to study immunomodulatory agents or antibodies.

This model, however, lacks robust T cell responses *in vivo*, including allograft rejection and sufficient T cell repopulation in the periphery, although there has been observation of T cell dependent antigen specific antibody responses [11] and islet allograft rejection [17]. This limitation presumably is attributed to MHC mismatch of the host mouse thymus and engrafted human APC in the periphery. Another limitation of this model is an absence of systemic lymphoid-like organoids, including Peyer's patches and the lymph nodes of human cells.

SCID-hu Mouse Model

An alternative approach to enhancing HLA restricted selection of a human T cell is providing a human thymus from the same donor of the human HSC. This mouse model is named SCID-hu, and was developed by co-implantation of human fetal liver and thymus into immunodeficient mice [18]. In this mouse, human thymocytes are selected on autologous thymic epithelium and HLA is restricted, resulting in excellent development of human thymocyte in the thymic graft. This model has been extensively used for studies of human thymopoiesis [19] and human immunodeficiency virus (HIV) infection [20, 21]. These mice, however, could not establish complete repopulation with multilineage hematopoietic cells in the periphery and lacked effective immune responses *in vivo*, even though human thymopoiesis is well achieved or human T cells show good function *in vitro*.

CHAPTER 41

What are Clinical Studies?

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Abstract: A clinical study, clinical trial, and clinical protocol are all concerned with a similar question. How does a new treatment affect patients?

Thru clinical studies, clinical researchers find new and better methods to address illnesses. A clinical trial protocol is used to manage a trial that is prepared by specialists. All clinical researchers are expected to strictly follow the protocol.

The aim of this review is to describe the Phase I, II, and III studies, and translational research for cancer tumors.

Keywords: Clinical studies, Phase I study, Phase II study, Phase III study, Translational study.

INTRODUCTION

A clinical study, clinical trial, clinical protocol are all concerned with a similar question. How does a new treatment affect patients? Thru clinical studies, clinical researchers seek new ways to address illnesses. Clinical studies include biomedical or behavioral research on human subjects designed to answer questions about biomedical or behavioral interventions (*e.g.*, drugs, treatments, *etc.*), and to generate safe and effective data.

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CLINICAL TRIALS

Clinical trials are studies that involve patients to test new methods to address diseases. Patients who are registered in cancer clinical trials have an opportunity to contribute to the knowledge base regarding cancer and help in the development of new cancer treatments. These investigations are conducted after information has been gathered that satisfies appropriate health authority and have ethics committee approval.

CLINICAL TRIAL PROTOCOL

A clinical trial protocol is used to manage a trial [1 - 4] and is prepared by experts. All study investigators are expected to strictly observe the protocol [4], which includes the scientific rationale, objective(s), design, methodology, statistical considerations, and organization of the planned trial [4]. The protocol also includes a precise study plan to guarantee the safety and health of the trial subjects, and provide an exact template for clinical researchers to conduct the trials. This protocol allows data to be combined across all researchers and sites, and informs study administrators. The format and content of clinical trial protocols are checked by pharmaceutical, biotechnology, or medical device researchers in Japan and have been standardized to follow the *Good Clinical Practice* guidance issued by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.

Medications found to be helpful and safe in test tubes and in animals, must also be proven safe and effective in humans before use by physicians in practice. Testing in humans is permitted only if volunteers understand the risks and benefits of taking part in the study. Investigators obtain informed consent to participate, which must be based on the volunteer's understanding of the research, including risks and benefits.

Additionally, volunteers may leave a research study at any time.

PHASES

Clinical trials are conducted in a series of steps called phases—each phase is designed to answer a separate research question. In a Phase I study, the

investigators examine a new drug with a small group of people for the first time to evaluate its safety, determine a safe dosage range, and identify side effects [1]. The aim of a Phase I trial is to determine whether a new drug is safe, the best way to give the new treatment, and whether signs are present that the cancer responds to the new treatment. The investigators increase the dose with each new cohort until they find the best dose for future testing. With each increasing dose, the investigators examine each patient to determine whether the patient is responding to the treatment. If they find that the treatment is safe, then it will move forward for study in a Phase II trial.

Phase II

In a Phase II study, the drug or treatment is given to a larger group of people to determine whether it is effective and to further evaluate its safety [2, 3]. This trial examines whether one type of cancer responds to the new treatment. Even though the aim is to determine whether the drug works, the investigators also watch for patient's side effects. If the new drug works, they may go on to study it in a Phase III trial.

Phase III

In a Phase III study, the drug is given to large groups of people to confirm its effectiveness, monitor side effects, compare it to commonly used treatments, and collect information that allows the drug to be used safely [4]. This trial examines whether a new treatment is better than a standard treatment. Phase III trials may include a considerable number of patients country or worldwide. Each patient enrolled in a Phase III clinical trial has a chance of being in one of the following groups: 1) Control group or 2) Study group.

In a Phase III study, the investigators do not know whether the new treatment is better than the standard treatment. Phase III studies compare the results of patients taking the new treatment to those taking the standard treatment. Usually, the studies move into Phase III only after a treatment seems to work in Phases I and II, and these clinical trials take many years to complete. Phase III trials are randomized and may include a double-blind method, and the investigators place participants into treatment groups at random.

CHAPTER 42

The Hospital Information System Using Two Separate Virtual Servers Connected to the Internet with Strong Security

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Abstract: To achieve both security and convenience of hospital information systems, we introduced two virtual servers; one with an Internet connection and the other using electronic medical records not connected to the Internet. Electronic medical records and other applications, such as mailer, Microsoft office products, and Internet browser, were ran by Citrix XenApp™, a product of an application virtualization. The virtual servers connected to the Internet and the virtual servers for the electronic medical records were isolated, and the interface between the two was strictly cutting off. Clients were based on the thin and thick client principles, and ordinary personal computers were allowed for applications not installed in the virtual servers. We set up personal data and unit data repositories in the virtual server that was connected to the Internet. Therefore, when users logged in using the contact type IC card to authenticate the active directory, they were able to operate both the electronic medical records and the shared data repositories anywhere in the hospital. Users could search the Internet even while writing electronic medical records, and the number of PCs connected to the electric medical records was increase significantly, which resulted in additional expansion of the blade servers. To develop the virtualization system of the HIS, stabilization of virtualization technology, virtual license fee of applications, and improvement of management capacity will be required.

Keywords: Client, Electronic medical records, Hospital information system,

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Internet, Virtual servers.

INTRODUCTION

The healthcare information system (HIS) has been operating using a closed system because of security problems in Japan [1]. Network connection operations have been separate from the HIS network to maintain patient privacy. However, a need has existed to search the Internet during patient examination to access drug information and clinical guidelines [2, 3]. We were unable to use the Internet while writing electrical health records (EHR), because the EHR system was operating in a client-server model within a closed system network. Therefore, physicians had to use two personal computers (PCs; not supplied by the hospital) connected to the different networks: the EHR network and the Internet. Many physicians have been registering patient information on their private PCs and USB flash drives, which could be transferred out of the hospital. Additionally, USB flash drive management has not been performed because of mixture of Windows and MAC PCs; therefore there has been continuing risk of patient information leakage [4].

In accordance with the HIS update in September 2011 at the National Hospital Organization Kure Medical Center, we intended to create a new environment with the ability to view the Internet even when writing the EHR (EG-MAIN GX, Fujitsu Co, Japan) without security problems. As attention to virtualization technology in the healthcare field has increased [5 - 7], we introduced an advanced virtualization system to search the Internet while writing EHR with secured privacy (Fig. **1**).

SERVER-BASED COMPUTING AND THIN CLIENTS

Server-based computing (SBC) and thin clients [8] is a technology whereby applications are executed on a server; not on client. Performance in server-based computing is better than that in a web application model or client server model, which require a lot of data to be sent back and forth between server and clients [9].

The EHR and non-EHR applications were installed on the two virtual servers, and

the interface between them was used to ensure security. The EHR application was installed on XenApp™, which is a type of application virtualization [10]. Non-EHR applications, such as radiology information system, laboratory system, transfusion system, pathology system, physiology system, and data warehouse, were ran on VMware™, which is a type of desktop virtual infrastructure [11]. The capacity of integrated virtual servers required from each unit system could be reduced significantly to three servers (from seven) with a cost reduction of 2.4 million yen.

Servers of non-updated unit systems were transferred and aggregated as physical servers; many of those distributed to various departments were integrated in the server room. The management software of physical and virtual servers was also introduced, which made it possible to visualize the actions of all servers.

The HIS network, which included the EHR and non-EHR systems, was not been able to connect to the Internet. On the other hand, the non-HIS network was connected to the Internet. Web browser software, mailer, and Microsoft Office products were installed in the virtual servers of the non-HIS network that was connected to the Internet.

As power density in blade servers [12] is higher than rack servers, it was not sufficient in a room with the usual air conditioner unit. Therefore, it became necessary to expand the air conditioner and repair of the server room.

ACTIVE DIRECTORY AND CONTACT TYPE IC CARD

A major feature of this system is the way it advanced server virtualization technology to deliver both the convenience of using different applications on thin clients and its high level of security using the type B integrated circuit (IC) cards [13] that were adopted from the basic resident registration cards used by local governments in Japan.

Security was ensured by active directory authentication with Single Sign-on (SSO) [14], which was based on two factors: authentication by both contact type IC card and password when users login [15]. When the contact type IC card is inserted into the card reader, the user is recognized as active. The user must

Part 3
CASE REPORTS

CHAPTER 43

Dermoscopy for Pigmented Skin Lesions: Four Case Reports

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Abstract: Dermoscopy is a useful tool to examine pigmented skin lesions. We use this method as a routine tool and have experienced many pigmented lesions. Here, we present four pigmented skin lesions (three are pigmented nevi and one is malignant melanoma) to demonstrate the usefulness of dermoscopy when making a clinical diagnosis of pigmented skin lesions.

Keywords: Dermoscopy, Malignant melanoma, Pigmented nevus, Routine tool, Skin lesion.

INTRODUCTION

Dermoscopy appeared more than 10 years ago. In Japan, it became popular after the procedure became eligible for health insurance remuneration in 2006. When dermatologists look at skin lesions, they apt to consider their histological features. Dermoscopy is the examination of skin lesions with a magnifying dermatoscope, which allows the dermatologist to inspect the skin lesions unobstructed by skin surface reflections. Under stronger lightning, dermoscopy allows for the inspection of dermal changes in lesions. The data obtained with the dermoscopy is greater than that by naked ocular observation, and they are useful for considering the histological features of the lesions. Dermoscopy is most useful when looking

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at pigmented skin lesions. We have significant experience with pigmented nevi (PN) and malignant melanoma (MM) cases using the HEINE DELTA 20 Plus Dermatoscope (Herrsching, Germany).

Pigmented nevi consist of nevus cells that are similar to melanocytes in feature and function. Pigmented nevi are divided into congenital and acquired lesions. Congenital lesions exist at birth, are relatively large in size, and are uniformly black in color. Acquired lesions are usually small in size (about several millimeters in diameter). In comparison, MM are melanocyte-derived malignant tumors and are well known to show very poor prognosis. To date, few MM cases have been reported in Japan; however, recent public concerns are increasing due to television coverage about MM. Misunderstandings about MM may occur when individuals obtain information from television programs, and some patients (adults and children) complain that all the pigmented lesions on their palms and soles may be MM. Other patients refuse excision of benign lesions. Taken together with these findings, dermoscopy is often used to differentiate skin lesions, and we use it as a tool to examine skin pigmented lesions. Here, we show its usefulness by presenting four cases.

CASE PRESENTATION

Case 1: A 9-year-old Japanese boy complained of a black-pigmented lesion on his left sole that appeared at 3 years old and increased gradually in size. His parents asked to excise it. On macroscopic examination, an 8 x 4mm-sized, border-clear, black nodule was detected on his left sole (Fig. **1A**). According to the ABCDE-criteria (A, asymmetry; B, border irregularity; C, color variegation; D, diameter; and E, evolution; by the American Cancer Society) for diagnosis of MM, this case was suspected to be a MM by naked ocular observation. However, dermoscopy revealed a typical parallel furrow pattern (Fig. **1B**), which is a typical finding of PN of the palm and sole, and showed pigmentation along with a skin groove structure [1]. We re-considered this case as PN of the sole, but performed excisional biopsy upon the parents' request. Histological diagnosis was a junctional nevocellular nevus, which was consistent with our clinical diagnosis done with dermoscopy.

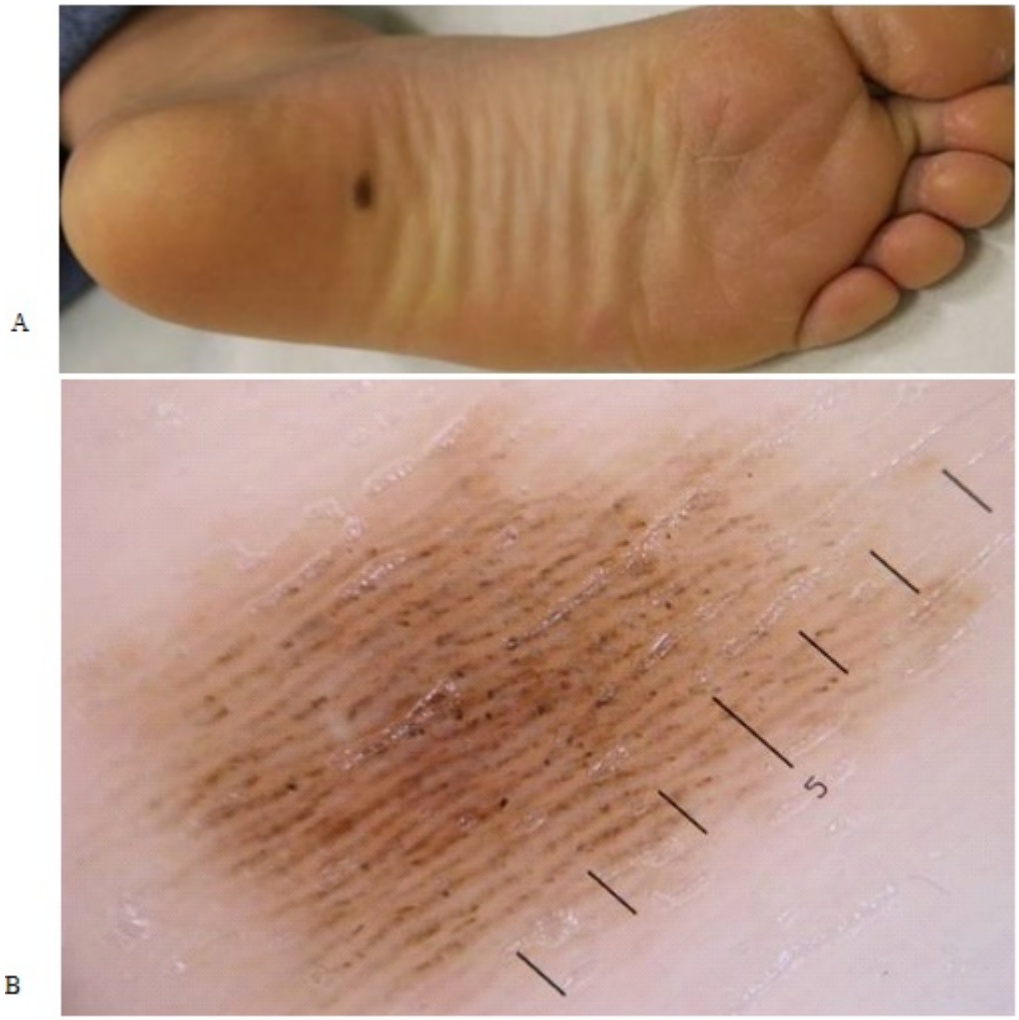

Fig. (1). (A) Black nodule on patient's left sole in case 1; **(B)** And dermoscopy findings of black nodule showing a parallel furrow pattern.

Case 2: A Japanese woman in her 80s complained of a 20-year standing black nodule on the internal side of her right foot. A doctor who saw the lesion recommended an excisional biopsy. She was referred to our department, and we found a 7 x 5 mm-sized, irregularly pigmented black nodule on the internal side of her right foot (Fig. **2A**). Dermoscopy revealed pigmentation along with a skin groove structure and another pigmentation that was orthogonal to the former one. This pigmentation is called a Lattice-Like pattern [2], which is a subtype of a

CHAPTER 44

A Case of Superior Sagittal Sinus Thrombosis Presented with Papilledema

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Abstract: This is a case report of superior sagittal sinus thrombosis (SSST), with bilateral papilledema as the initial manifestation.

A 65-year-old male was referred to us with bilateral decreased vision and recurrent blackouts associated with postural change. Although his intracranial pressure was increased, an intracranial space-occupying lesion was not detected by computed tomography (CT). Magnetic resonance imaging (MRI) with a fluid-attenuated inversion recovery (FLAIR) technique and magnetic resonance venography (MRV) findings revealed a thrombosed superior sagittal sinus. Anticoagulation therapy with heparin was started and recurrent blackouts disappeared one month thereafter. Thoracoabdominal CT examination revealed an infiltrative shadow in the right inferior lobe of the lungand was diagnosed as squamous cell carcinoma from biopsy by bronchoscopy. Bilateral papilledema gradually decreased over an 18-month observation period after anticoagulation therapy.

MRV was useful in the diagnosis of SSST in this case of bilateral papilledema with increased intracranial pressure.

Keywords: Cerebral venous sinus thrombosis, Magnetic resonance venography, Papilledema, Recurrent blackouts, Squamous cell carcinoma.

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INTRODUCTION

Cerebral venous sinus thrombosis (CVST) is a relatively uncommon condition. The initial diagnosis may be mistaken because the symptoms and clinical courses are highly variable.

We report here on a case of superior sagittal sinus thrombosis (SSST), with bilateral papilledema as the initial manifestation, where magnetic resonance venography (MRV) was useful in the diagnosis.

CASE PRESENTATION

A Japanese male in his sixties with past medical history of diabetes mellitus and hypertension had recurrent blackouts associated with postural change for a one month period, and was referred to our outpatient clinic on August 24, 20XX. He had been in need of the long-term care system because of walking difficulty due to diabetic neuropathy and lumbar spinal canal stenosis.

On examination, his best-corrected visual acuity was 0.5 RE (right eye) and 0.3 LE (left eye). The intraocular pressure was 17mmHg (RE) and 18mmHg (LE). Eye movement was not disturbed in both eyes. Pupillary reactions were normal. Moderate nuclear cataracts (Emery-Little grade 2) were found in both eyes. Funduscopic examination revealed bilateral papilledema with dilated retinal veins (Fig. **1**). Results of Goldmannperimetry showed enlargement of Mariotte's blind spot in both eyes (Fig. **2**). Enhanced computed tomography (CT) head scan showed no intracranial space-occupying lesion.

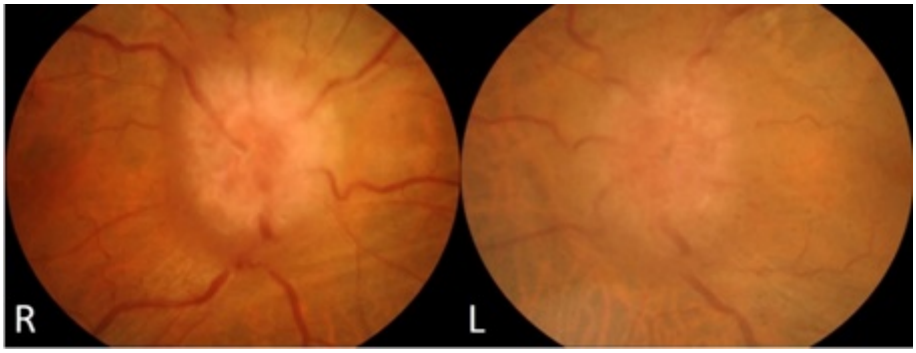

Fig. (1). Marked papilledema with dilated veins in both eyes.

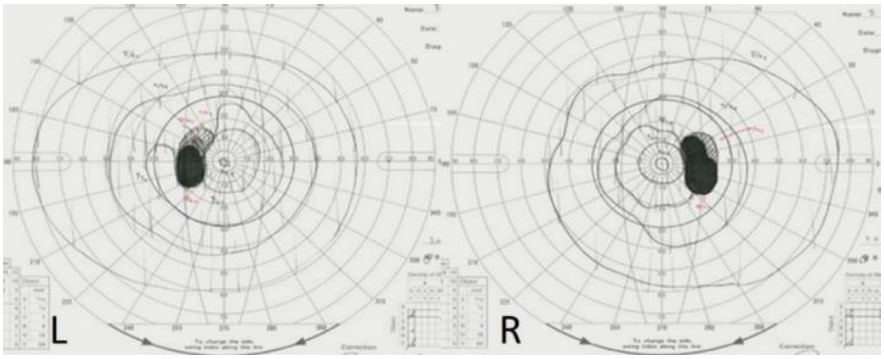

Fig. (2). Mariotte's blind spots enlarged in both eyes.

He was referred to the neurological department. On August 28, a spinal fluid examination performed by lumbar puncture revealed elevated opening pressure (280 mmH_2O), with slightly elevated protein (41 mg/dL) and glucose (79 mg/dL) levels. Laboratory studies showed elevated blood glucose (123mg/dL) and HbA1c (6.4%), and a coagulation profile showed elevated D-dimer (1.2µg/mL) and fibrinogen (469 mg/dL). Magnetic resonance imaging (MRI) with a fluid-attenuated inversion recovery (FLAIR) technique (Fig. **3A**) and magnetic resonance venography (MRV) findings (Fig. **3B**) revealed a thrombosed superior sagittal sinus.

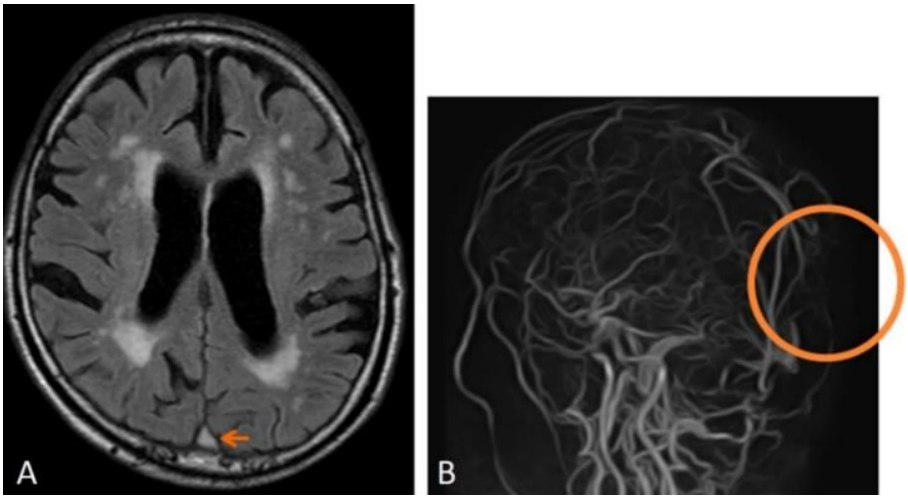

Fig. (3). MRI with FLAIR (**A**) and MRV (**B**, in circle).

CHAPTER 45

Verification of New Method for Stroke Volume Assessment by Echocardiography: Preliminary Study Report

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Abstract: Guidelines for the valuation of valvular heart disease by echocardiography recommend the quantification of severity. For accurate quantification, it is necessary to assume the mitral annulus (MA) area to calculate mitral stroke volume (SV). We applied and preliminarily verified a new method to calculate the MA area.

A Japanese man in his 60s without any abnormal cardiac findings including regurgitation in the aortic or mitral valve was simultaneously evaluated by a conventional and new method. The MA area was calculated by using a conventional method with an apical 4 chamber view (4CV) and apical 2 chamber view (2CV), and a new method with an apical commissure-commissure view (CCV) and apical 3 chamber view (3CV). Mitral SV was calculated from the MA area values by both methods and compared with left ventricular SV (LVSV) by using a modified Simpson method.

MA areas estimated by the new method were smaller than that those estimated by the conventional method. Mitral SV estimated by the new method was closer to LVSV than that estimated by the conventional method.

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The new echocardiographic method to calculate an MA area with apical CCV and 3CV may be more exact and useful than the conventional method. The results in this case report suggest that further evaluation at a larger scale may yield promising results.

Keywords: Echocardiography, Mitral annulus area, Stroke volume assessment, Valvular heart disease, Valvular regurgitation volume.

INTRODUCTION

Recently, quantitative assessment by color Doppler echocardiography, in addition to a qualitative assessment, has become more important as an objective indicator for valvular heart disease severity [1, 2]. Accurate estimation of the mitral annulus (MA) area is important to calculate mitral stroke volume and valvular regurgitation volume for diagnosis. The conventional method, however, to estimate MA area and mitral stroke volume (SV) is not accurate and a new method has been recommended [3]. In this study, we applied this new method to one healthy person and verified its efficacy by comparing mitral SV and left ventricular SV (LVSV).

SUBJECT AND METHODS

A healthy Japanese man in his sixties was the subject in this preliminary study. He did not have any heart disease including regurgitation in the aortic or mitral valve. He was simultaneously evaluated using both the conventional and new method with an echocardiograph (SSD-6500, Hitachi-Aloka Medical, Japan) and sector probe. The MA area was calculated by the following formula; π X half of major axis length X half of minor axis length, obtained either by using the conventional method with an apical 4 chamber view (4CV) and apical 2CV or the new method with an apical commissure-commissure view (CCV) and apical 3CV (Fig. **1**). Using Doppler and two-dimensional echo data, mitral SV was calculated as the MA area (cm^2) times the area under the velocity curve (velocity time integral; VTI). LVSV were calculated from diastolic and systolic volumes using a modified Simpson's method.

RESULTS

The MA area estimated by the conventional method was 8.99 cm^2, while that estimated by the new method was 7.15 cm^2, which was smaller than that estimated by the conventional method. Mitral SVs estimated by the new and conventional method were 71 ml and 88 ml, respectively. Theoretically, mitral SV should be equal to LVSV without any valvular regurgitation, thus the mitral SV calculated by each method was compared with LVSV. LVSV was 68 ml and much closer to the mitral SV estimated by the new method than that estimated by the conventional method.

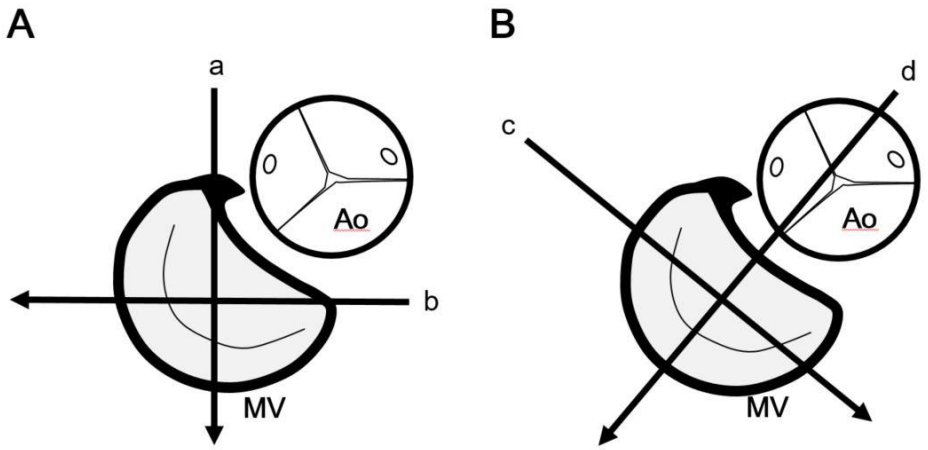

Fig. (1). (A) Angle of the ultrasound probe for a conventional method; **(B)** New method. Apical 2 chamber view (2CV) and apical 4CV (a and b, respectively) in conventional method and apical commissure-commissure view and apical 3CV (c and d, respectively).

DISCUSSION AND CONCLUSION

According to Japanese Circulation Society guidelines, a quantitative assessment by echocardiography is recommended for determination of therapeutic strategy. For a quantitative assessment, mitral SV is necessary and is calculated from the MA area. In Japan, the MA area has been assumed by the long axis from an apical 4CV and short axis from an apical 2CV. However, these cross-sections do not reflect an exact anatomical MA area [3]. Supposing the MA area is an ellipse, the exact long axis and short axis can be gained from an apical CCV and apical 3CV,

CHAPTER 46

Eosinophilic Pancreatitis with Psoriasis Vulgaris

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Abstract: Eosinophilic pancreatitis (EP) is a rare disease characterized by eosinophilic infiltration of pancreatic parenchyma. Although the etiology of EP is not fully understood, there may be some allergic mechanisms related to the disease. Here we report on an EP patient with psoriasis vulgaris.

A 47-year-old man was admitted with cardiac space pain and a feeling of abdominal compression. He had a history of psoriasis vulgaris treated with steroid ointment for the past 30 years. Laboratory findings revealed elevated serum titers amylase. An abdominopelvic computed tomography scan showed a cystic lesion 45 mm in diameter localized in the pancreatic tail. He was diagnosed with pancreatic pseudocyst and conservative medical treatment was initiated with antimicrobial drugs and gabexate mesilate. Whenever meals were taken, pancreatitis aggravation returned. Eosinophilic leukocytosis occurred during steroid administration. Pseudoaneurysm in the splenic artery solution part occurred and a splenic artery embolism was performed in interventinal radiology. The patient underwent distal pancreatectomy. The operation specimen revealed eosinophilic infiltration.

Despite the unusual occurrence of EP, it should be considered in the differential diagnosis of patients with allergic disease or with an eosinophillia group presenting pancreatitis. In such cases, EUS-FNA or laparoscopic biopsy should be mandatory to

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avoid unnecessary surgical operation.

Keywords: Distal pancreatectomy, Eosinophilic pancreatitis, Pancreatic pseudocyst, Psoriasis vulgaris, Steroid.

INTRODUCTION

Eosinophilic pancreatitis (EP) is a very rare disease characterized by eosinophil infiltration of pancreatic parenchyma [1 - 4]. Patients usually complain of abdominal pain. The prognosis of EP is comparatively good and medical treatment is effective [1]. However, the diagnosis is difficult and cases of false positive pancreas resection due to this disease have been reported [1]. We report on a case of EP with psoriasis vulgaris and discuss the case in light of information in the literature.

CASE REPORT

A 47-year-old Japanese man was admitted to our hospital for cardiac space pain and a feeling of abdominal compression. Computed tomography (CT) scan revealed a cystic lesion 45 mm in diameter localized in the pancreatic tail and several low-density areas in the pancreatic body (Fig. **1A**). He was diagnosed with a pancreatic pseudocyst and hospitalized. He had a history of psoriasis vulgaris treated with steroid ointment for the past 30 years. Drinking history was evaluated as at an opportunity grade. Smoking history was for 25 years at 30 cigerettes per day. Conservative medical treatment was initiated with the antimicrobial drugs and gabexate mesilate. The patient left the hospital after 22 days. One month later, the patient had a high fever and upper abdominal pain, and a CT scan revealed suspect perforation of a pancreatic pseudocyst (Fig. **1B**). He was admitted to our hospital. Complete blood count showed WBC 22,000/μL (Eosinophils, 0.9%), hemoglobin 14.9 g/dl, and platelet 39.9 g/μl. The serum biochemistry showed amylase 273 IU/L, total bilirubin 1.8 mg/dl, CRP 10.15 mg/dl, and CA19-9 105 U/ml. The patient underwent primary care that comprised of a fast and antibiotic medication, and the pancreatitis improved. However, upon oral consumption, pancreatitis aggravation recurred. And, due to the skin, central venous catheter fever also recurred.

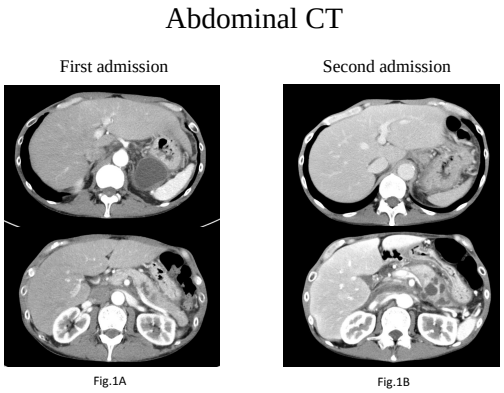

Fig. (1). (A) Abdominopelvic computed tomography (CT) scan. CT image shows a cystic lesion 45 mm in diameter localized in the pancreatic tail and several low-density areas in the pancreatic body. **(B)** Pancreatic pseudocyst suspected of perforating.

About 70 days later after the second admission, there was a rise in white corpuscles (9,500~53,300/μL) and eosinophilic leukocytosis (1,910~14,500/μL), which were in agreement with aggravation of the cutaneous symptom of psoriasis vulgaris. Ventricle-wall thickening was suspected after the cardiac ultra-sound (Fig. **2**). For myocarditis accompanied by eosinophilic leukocytosis, steroid administration (predonine 20 mg /day) was given, and the number of eosinophils normalized.

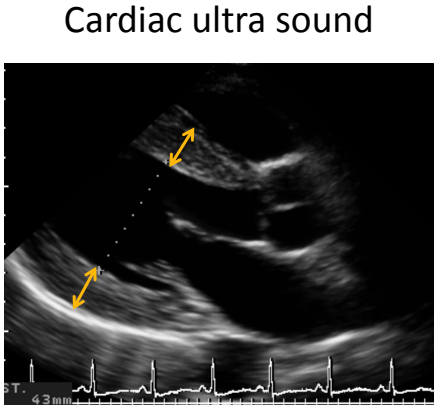

Fig. (2). Cardiac ultra-sound shows ventricle-wall thickness.

Approximately 87 days later after the second admission, the patient had acute abdominal pain and the serum amylase elevated to 366 IU/L. CT revealed the

CHAPTER 47

Acute Renal Failure Due to Ethylene Glycol Poisoning: A Case Report

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Abstract: Acute renal failure caused by ingestion of ethylene glycol during a suicide attempt is rare. In this case report, a Japanese man ingested ethylene glycol in a suicide attempt and was transported to our hospital. He complained of severe nausea and blood tests revealed metabolic acidosis, and elevated white blood cell count, creatinine and BUN levels. Hemodialysis was implemented four hours after the ethylene glycol was ingested, and urine volume increased to 1,500 mL/day on the ninth day after admission. Creatinine clearance, however, did not improve. A renal biopsy performed on the 45th day revealed numerous collapsed glomerulus, cortical infarction, and swelling of the renal tubular epithelial cells. Hemodialysis has been recommended for the treatment of ethylene glycol poisoning in the presence of severe metabolic acidosis that is unresponsive to therapy.

Keyword: Acute renal failure, Ethylene glycol, Hemodialysis, Metabolic acidosis, Poisoning.

INTRODUCTION

Although there have been many reports of acute renal failure caused by ethylene glycol poisoning, only a few have resulted in maintenance hemodialysis. We report here on a case that resulted in maintenance hemodialysis after ingestion of ethylene glycol during a suicide attempt.

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CASE REPORT

A Japanese man in his 30s with no past medical history ingested 150 mL of radiator fluid (ethylene glycol) during an attempted suicide. He was transported to our hospital one hour after he had ingested the radiator fluid. His consciousness level was alert (Japan coma scale 0) and he complained of severe nausea. His respiratory rate was 20/min, heart rate was 50/min sinus, blood pressure was 154/92 mmHg, SpO2 was 99% (room air) and body temperature was 35.0°C. There was no neurologic manifestation. There was no abdominal pain or signs of peritoneal irritation. Venous blood gases showed metabolic acidosis with pH 7.256, PO_2 45.7 torr, PCO_2 32.0 torr, HCO_3^- 13.7 mmol /L, BE -12 mEq/L, and anion gap 19 mEq/L. Blood examination revealed elevated levels of white blood cells at 21,000 /μL, Cre and BUN with values of 9.6 mg/dL and 61 mg/dL, respectively. Abdominal plain CT showed only mild renal swelling, and there were no other abnormalities. He was diagnosed with acute renal failure from ethylene glycol poisoning, and was admitted to an intensive care unit.

After admission, his urine volume was found to be very low in volume. Although 1,000 ml infusion of extracellular fluid and ethanol as an antimetabolite was undertaken, oliguria counting for 10 mL/h of urine volume continued. As such, we started hemodialysis four hours after he had ingested the ethylene glycol to remove glycolate, and subsequently the ethylene glycol levels lowered. On the ninth day after admission, the urine volume increased to 1,500 mL/day. However, creatinine clearance changed at around 4 mL/min and did not improve. A renal biopsy performed on the 45th day after admission showed numerous collapsed glomeruli, cortical infarction, and swelling of the renal tubular epithelial cells (Fig. **1**). Hemodialysis secession was considered to be difficult, so that the patient underwent vascular access in his left forearm and hemodialysis was continued.

DISCUSSION

Ethylene glycol is used extensively in antifreeze and batteries. When it is ingested, it is metabolized as glycoaldehyde by alcohol dehydrogenase in the liver into glycolate, glyoxylate and oxalic acid [1]. Metabolic acidosis and organ dysfunction primarily result from generation of glycolic and oxalic acid from

metabolism of ethylene glycol. The mortality of ethylene glycol poisoning varies, ranging from 1 to 22% [2, 3].

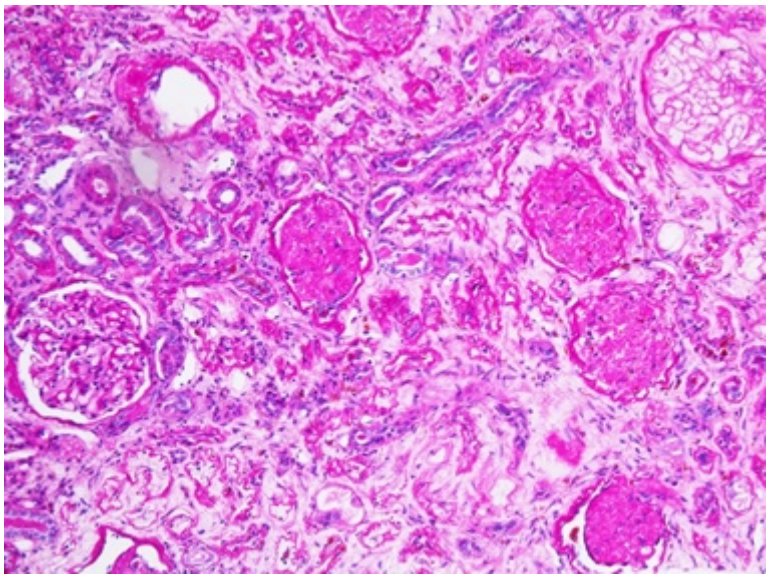

Fig. (1). Histological findings of renal biopsy showing collapsed glomeruli, cortical infarction, and swelling of the renal tubular epithelial cells (PAS, Obj 10X).

Ethylene glycol has a potent influence on neurology, cardiovascular and renal functions. Typically, in the first 12 to 24 hours after ingestion, a mild disturbance of consciousness may occur. Following neurology symptoms, heart failure may appear. Last, acute renal failure develops [4]. In this case, the patient showed acute renal failure after severe nausea with neurologic abnormalities. In particular, there was severe metabolic acidosis and oliguria due to reduced renal function. The factors involved in mortality include the time to severe metabolic acidosis and treatment initiation [4].

The American Academy of Clinical Toxicology recommends that ethanol or fomepizole should be given in the presence of an ethylene glycol level > 20 mg/dL or with a documented history of potentially toxic amounts of ethylene glycol and serum osmolal gap > 10 mOsm/L or history or strong clinical suspicion of ethylene glycole poisoning and two of the following abnormalities: arterial pH < 7.3, serum bicarbonate concentration < 20 mEq/L, osmolal gap > 10 mOsm/L,

CHAPTER 48

Intraductal Papilloma of the Breast with Necrosis Due to An Infarction: A Case Report

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Abstract: An infarcted intraductal breast tumor was excised from a woman in her 60s. The tumor was reduced in size from 15 x 8 mm to 6 x 6 mm one month after the fine needle aspiration biopsy. The necrosis was considered to be caused by an infarction and an immunohistohemical analysis revealed positive immunoreactivity of smooth muscle actin of myoepithelial cells that indicated a two-cell layer of tumor cells in the necrosis. Although this necrotic tumor was diagnosed as an infarcted intraductal papilloma by histological examination, its diagnosis was more difficult as it showed partial necrosis by cytological examination alone.

Keywords: Infarction, Intraductal papilloma, Myoepithelial, Necrosis, Smooth muscle actin.

INTRODUCTION

Necrosis is one suggestive finding for malignancy in aspiration cytology of the breast. However, when necrosis is caused by an infarction, which sometimes happens in benign lesions, differential cytological diagnosis is difficult. This is the

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case whether it is benign or malignant. Further, there are no definite findings can be obtained by a routine examination. Here, we present a case of intraductal papilloma of the breast with necrosis due to an infarction.

CASE

A Japanese woman in her 60s had a medical examination of her breasts with mammography that revealed a tumor of 15 x 8 mm in size in her left breast (Fig. **1**).

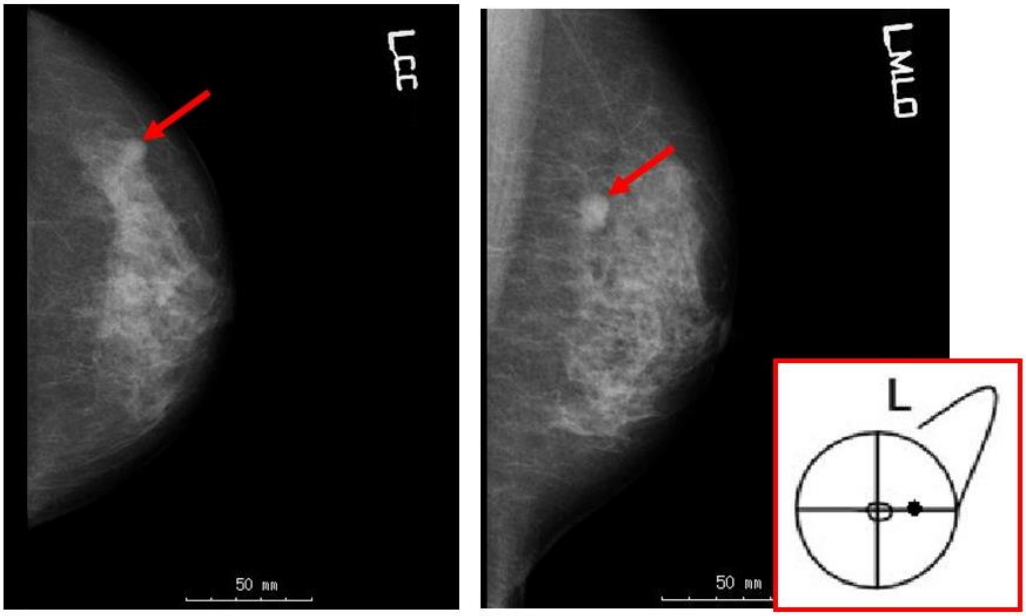

Fig. (1). Mammography findings indicate a nodular lesion (red arrow) in the left breast.

Fine needle aspiration cytology (FNAC) from the breast tumor was undertaken, but gave no conclusive diagnosis. One month after the FNAC, the tumor size, as measured with an ultrasound echo-gram, reduced to 10 mm in size just before the excision, and an excisional biopsy of the tumor was performed.

CYTOLOGICAL FINDINGS

FNAC from the breast tumor showed a few epithelial cells and some necrosis in the background of the hemorrhage (Figs. **2** and **3**). These epithelial cells were

relatively small in size, cohesive to each other, and arranged in a papillary configuration (Fig. **4**).

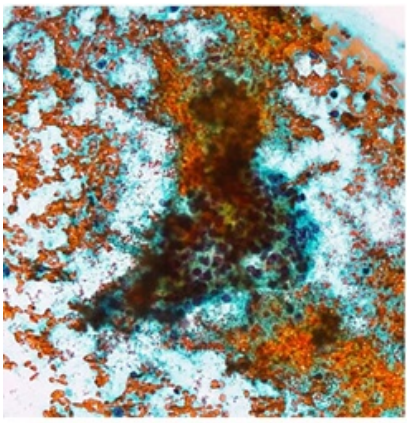

Fig. (2). FNAC findings (Pap, obj 20X).

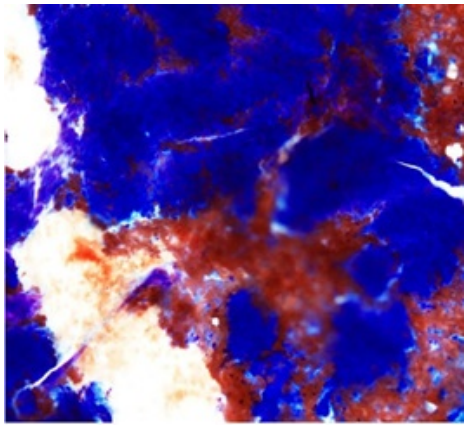

Fig. (3). FNAC findings (Giemsa, obj 10X).

CHAPTER 49

Imprint Cytology of Extrarenal Retroperitneal Angiomyolipoma: A Case Report

Daiki Taniyama[1], Kazuya Kuraoka[1,], Atsushi Yamaguchi[2], Masahiro Tanemura[3], Takuo Ito[4] and **Kiyomi Taniyama[1,*]**

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Abstract: Renal angiomyolipoma (AML) is recognized as a benign renal tumor composed of atypical blood vessels, smooth muscle and fat tissue, and constitutes about 1% of all renal masses. In contrast, extrarenal AMLs are extremely rare tumors of which there have been less than 70 reported cases since they were first described in 1982. We present the case of a 65-year-old female with a retroperitoneal extrarenal mass. Histopathological examination and HMB-45 staining revealed the mass to be an extrarenal AML.

A 65-year-old female presented with a 2cm mass next to the pancreas head during a follow-up CT scan one year after chemotherapy for malignant lymphoma. Diagnosis of the tumor was not possible with EUS-FNA cytology. The tumor was considered to be a solid-pseudopapillary neoplasm (SPN) or endocrine tumor of the pancreas. Subsequently, surgical resection of the tumor was performed.

Gross microscopic examination of the tumor, which was located in the retroperitoneum and was separated from the pancreas, showed histologically tortuous thick blood vessel with bundles of smooth muscle. Mature adipose tissue was also detected. Imprint cytology of the cut surface of the tumor showed atypical cells which had spindle-

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shaped outlines, nuclear enlargement and hyperchromasia. HMB-45 staining performed on the larger lesion was positive, which is characteristic of AMLs.

Extrarenal AMLs are rare and occur most commonly in the liver. Lesions in the retroperitoneum require definitive diagnosis since they can mimic other benign and malignant retropelitoneal tumors, which must be differentiated.

Keywords: Angiomyolipoma, Blood vessel, Fat tissue, Renal tumor, Smooth muscle.

INTRODUCTION

Renal angiomyolipoma (AML) is recognized as a benign renal tumor composed of atypical blood vessels, smooth muscle and fat tissue, and constitutes about 1% of all renal masses [1]. In contrast, extrarenal AMLs are extremely rare tumors of which there have been less than 70 reported cases since they were first described in 1982. We present the case of a 65-year-old female with a retroperitoneal extrarenal mass. Histopathological examination and HMB-45 staining revealed the mass to be an extrarenal AML.

CASE

A 65-year-old female presented with a 2cm mass next to the pancreas head during a follow-up CT scan one year after chemotherapy for malignant lymphoma (Fig. **1**). Diagnosis of the tumor was not possible with EUS-FNA cytology because of inadequate materials obtained.

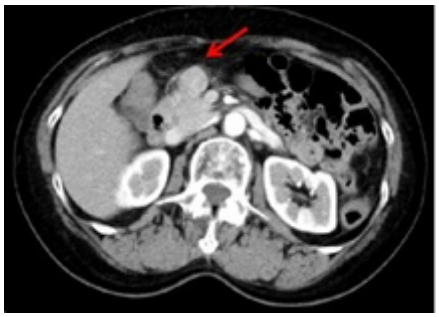

Fig. (1). Abdominal CT finding (Arrow indicates the tumor).

The tumor was considered clinically to be a solid-pseudopapillary neoplasm (SPN) or endocrine tumor of the pancreas. Subsequently, surgical resection of the tumor was performed (Fig. **2**).

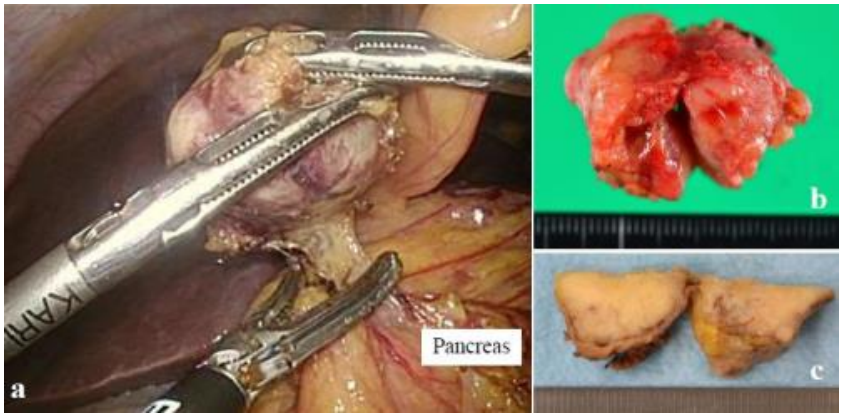

Fig. (2). Macroscopic findings showing, (**a**) features of tumor during the operation, (**b**) surface of resected tumor (5.7g, 3.0 x 2.0 x 1.8cm), and (**c**) cut surface of the tumor after formalin-fixation.

Histological Findings

The tumor was located in the retroperitoneum and separated from the pancreas. It measured 3.0 x 2.0 x 1.8cm in size and was 5.7g in weight. It showed histologically tortuous thick blood vessels, and irregularly arranging bundles of smooth muscle (Fig. **3**). Mature adipose tissue was also detected in a small part (Fig. **4**). HMB-45 staining performed was positive for smooth muscle cells, which is characteristic of AMLs (Fig. **5**).

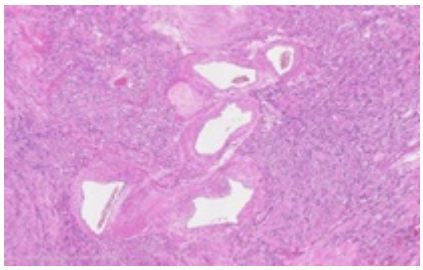

Fig. (3). Microscopic findings of the tumor showing tortuous thick blood vessels and irregularly arranging bundles of smooth.

CHAPTER 50

Internal Coil Trapping of a Ruptured PICA-Involved-Type Vertebral Artery Dissecting Aneurysm: A Case Report

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Abstract: Postoperative medullary infarctions have been reported as a poor prognostic factor after internal coil trapping of a ruptured vertebral artery dissecting aneurysm (VA-DA). We succeeded in treating a ruptured VA-DA involving the origin of the posterior inferior cerebellar artery (PICA) without critical medullary infarctions. We discuss the reasons why we were able to avoid critical medullary infarctions in this case report.

A 42-year-old Japanese man presented with a sudden onset of left sided headache. He also reported a history of neck pain suddenly occurring two days prior while playing the drum. He had a re-ruptured left VA-DA involving PICA. The re-ruptured VA-DA was initially treated by endovascular bleeding point coil embolization. Dejerine syndrome occurred as a complication of the initial treatment. The neurological symptoms were temporary and the patient made a recovery from the syndrome within a week. MRA revealed recanalization on day 16, and re-bleeding from the ruptured VA-DA was identified on CT scanning on day 20, which was treated by endovascular proximal coil embolization. Postoperative infarction was noted only in the small area of the left cerebellum. The patient showed marked improvement. On one-year follow-up, the patient remains in good clinical condition (Score 1 on modified Rankin scale).

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The bleeding point coil embolization and proximal coil embolization were not performed simultaneously in this case. Decreasing the length of the coil embolization could save the perforating branches.

Keywords: Endovascular treatment, Internal coil trapping, Medullary infarction, Subarachnoid hemorrhage, Vertebral artery dissection.

INTRODUCTION

Ruptured vertebral artery dissecting aneurysm (VA-DA) is recognized as one of the causes of subarachnoid hemorrhage (SAH) or posterior circulation ischemia in young or middle-aged adults [1]. In addition, a risk for recurrent hemorrhage has been reported as ranging from 30% to 70% with a high mortality rate estimated at 46% [2, 3]. Based on these facts, an endovascular approach has been proposed as the first line therapy to minimize the risks of re-bleeding along with the development of new devices.

In comparison, postoperative medullary infarctions have been reported as a poor prognostic factor after internal coil trapping of a ruptured VA-DA [4, 5]. However the reasons for these cases have not been fully examined. Our team succeeded in treating a re-ruptured VA-DA involving the origin of the posterior inferior cerebellar artery (PICA) without critical medullary infarctions. We discuss the reasons why we were able to avoid critical medullary infarctions in this case report.

CASE PRESENTATION

Identification and History of Present Illness

A 42-year-old Japanese man presented with a severe sudden onset of left sided headache. He also reported a history of sudden neck pain on the same side occurring two days previous while playing the drum. Brain CT showed SAH with the focus on the posterior cranial fossa and brain MRA revealed a left VA-DA. Because these findings and the patient's history suggested re-bleeding from the VA-DA, blood pressure control and pain management was started immediately. However, he developed an acute progressive severe headache, clouding of

consciousness, and respiratory arrest while we were preparing the emergency endovascular therapy. Intubation was performed promptly and respiratory management was started. Brain CT suggested a second re-rupture (Fig. **1**) and emergency angiograms of the left vertebral artery (VA) revealed a PICA-involving type of left VA-DA (Fig. **2**).

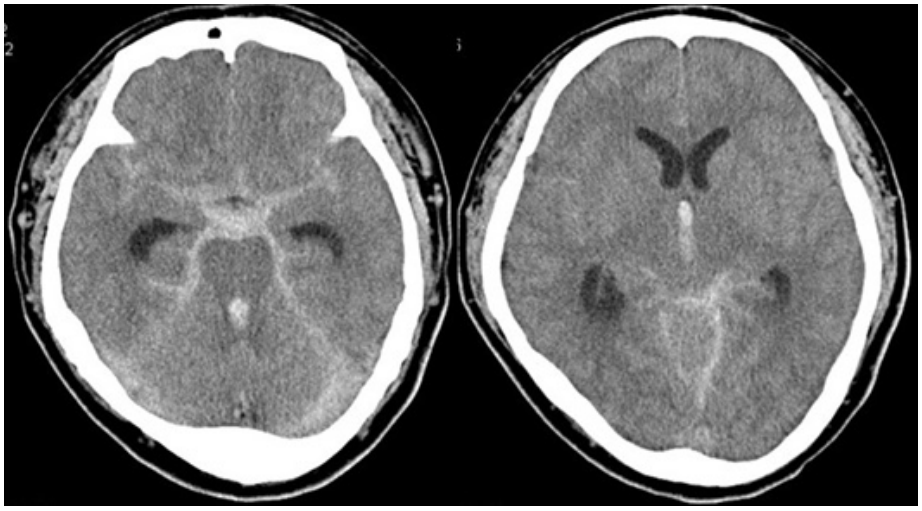

Fig. (1). Brain CT showing subarachnoid hemorrhage at the point of second re-bleeding from the ruptured vertebral artery dissecting aneurysm.

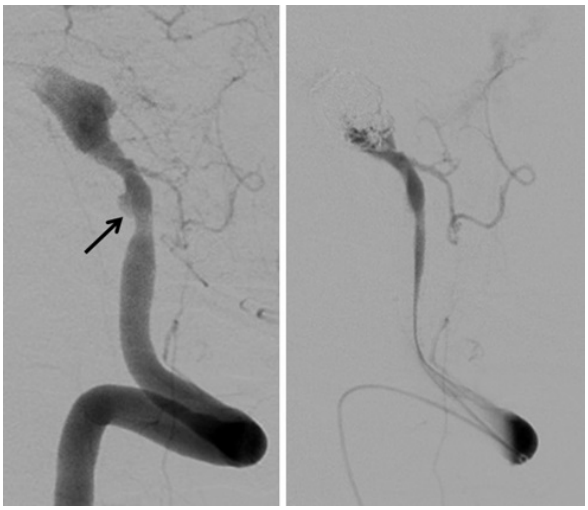

Fig. (2). Left vertebral artery angiogram showing the posterior inferior cerebellar artery-involving type of left vertebral artery dissecting aneurysm that was treated by endovascular bleeding point coil embolization.

Part 4
SHORT REPORTS

CHAPTER 51

An Approach for More Effective Detection of *Clostridium Difficile* in Patients

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Abstract: Stool samples from 432 patients with suspected for *Clostridium difficile* (CD) infection were tested with the novel kit, COMPLETE, which was evaluated for the efficacy in detecting glutamate dehydrogenase (GDH), and cultures were then re-evaluated to determine necessity.

CD positive samples by culture and GDH positive samples by COMPLETE were 71 (16.4%) and 80 (18.5%) of 432 samples, respectively. Sensitivity and specificity of COMPLETE for detection of GDH were 100% and 97.5%, respectively. The toxin types of 71 samples were toxin A+B+ in 45, toxin A-B+ in 21, and toxin A-B- in five. In comparison, the toxin types of 44 samples whose toxin was detected by COMPLETE were toxin A+B+ in 31, and toxin A-B+ in 13. The sensitivity and specificity of COMPLETE for detection of toxin were 66.7% and 100%, respectively.

The combined use of COMPLETE and culture of suspicious CDI samples is beneficial for prompt detection of CD.

Keywords: *Clostridium Difficile*, Glutamate dehydrogenase, Infection, Stool, Toxin.

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INTRODUCTION

Clostridium difficile (CD) is known to cause drug-induced diarrhea, and treatment of CD is an important issue for infection control in a hospital [1]. In a routine examination, CD toxin can be detected from the stool of a patient using a prompt detection kit. However, the detection rate of this kit is not high enough to diagnose CD infection (CDI) and some CDI may not be identified. At our center, in addition to using the detection kit, we also culture stool samples suspected of having CDI.

Recently, a novel kit named COMPLETE (C. DIFF QUIK CHEK COMPLETE®; Alere Inc., USA) was made available for CDI and can promptly detect glutamate dehydrogenase (GDH), and toxin A and B simultaneously.

METHOD

We evaluated the efficacy of COMPLETE for detecting GDH, and re-evaluated the necessity of culturing suspicious CDI samples using 432 stool samples from patients who complained of diarrhea between June and December 2012 at our hospital. Stool samples were cultured with CCMA-EX agar (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) for 48 hours at 37ºC. Identification of CD was tested with a Rapid ID 32A system (Sysmex bioMeriux Co. Ltd., Tokyo, Japan) when necessary. Genes of toxin A and B were analyzed using colonies cultured in CCMA-EX agar. NK9-NK11 primer (*tcd*A-PCR) for toxin A and NK104-NK105 primer (*tcd*B-PCR) for toxin B were used for PCR analysis.

RESULTS

Table **1** shows the relation between GDH detected by COMPLETE and data from culture in 432 stool samples from patients suspected of having CDI with diarrhea. Of these, CD positive samples by culture were 71 (16.4%), and GDH positive samples by COMPLETE were 80 (18.5%). Therefore, the sensitivity and specificity of COMPLETE for detection of GDH were evaluated at 100% and 97.5%, respectively. Nine samples with discrepant results were from patients who were treated with Vancomycin. Table **2** shows the relation between toxin detected in stool samples and data by culture. The toxin types of 71 samples that were CD

positive by culture were toxin A+B+ in 45, toxin A-B+ in 21, and toxin A-B- in five. In comparison, the toxin types of 44 samples whose toxin was detected by COMPLETE were toxin A+B+ in 31, and toxin A-B+ in 13. The sensitivity and specificity of COMPLETE for detection of toxin were counted as 66.7% and 100%, respectively. All isolated colonies were demonstrated to be positive for GDH and toxin (Table **3**).

Table 1. Comparison of *Clostridium difficile* detected by culture and GDH in stool.

GDH by COMPLETE	CD by culture			COMPLETE	
	Positive (%)	Negative (%)	Total (%)	Sensitivity (%)	Specificity (%)
Positive	71(16.4)	9(2.1)	80(18.5)		
Negative	0	352(81.5)	352(81.5)	100	97.5
Total	71(l6.4)	361(83.6)	432(100)		

GDH, glutamate dehydrogenase; CD, *Clostridium difficile*.

Table 2. Comparison between Toxin detected and *Clostridium difficile* cultured in stool.

Toxin by COMPLETE	Number of samples with toxin by culture			Sensitivity (%)	Specificity (%)
	A+B+	A-B+	A-B-		
Positive	31	13	0		
Negative	14	8	5	66.7	100
Total	45	21	5		

CONCLUSION

The combined use of COMPLETE and culture of suspicious CDI samples is beneficial for prompt detection of CD (Fig. **1**) [2].

CHAPTER 52

Quality Assurance of Immunohistochemistry for Breast Cancers using a Whole-slide Imaging System, Specified Software and Cell Lines

Miho Yoshida-Tanaka[1], Junichi Sakane[1], Yoshiko Kimura[2], Yoshimi Shitakubo[2], Kazuya Kuraoka[1] and **Kiyomi Taniyama[1,2,*]**

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Abstract: An auto-stainer (AS), whole-slide imaging system (WSI), and computer software were used as quality assurance tools for breast cancer immunohistochemistry (IHC).

Cell lines, such as MCF-7 for estrogen receptors and progesterone receptors, and SK-BR-3 for Topo2a, were co-stained with samples as IHC control.

Combinatory usage of AS, WSI, computer software and cell lines can reduce deviation in each IHC step and is beneficial for IHC quality assurance.

Keywords: Auto-analysis, Breast cancer, Immunohistochemistry, Quality assurance, Whole slide image.

INTRODUCTION

Currently, tissue samples are obtained and retained at a local institute are then

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used as control for immunohistochemistry (IHC) of breast cancer. However, tissue samples may vary when cutting many slides and tissue volume is usually small, such that there is a limitation in the number of slides available as for IHC control. This indicates the necessity for establishing universal control samples for IHC.

METHOD

To identify the usefulness of auto-analysis system composed of an auto-stainer (AS), whole-slide imaging system (WSI), and computer software as a quality assurance tool for breast cancer IHC, a Benchmark XT (Roche, Basel, Switzerland) as AS, NanoZoomer 2.0-HT (Hamamatsu photonics, Hamamatsu, Japan) as WSI, software (Genie/Aperio)-equipped computer and cell lines (Pathology research Inc., Toyama, Japan); MCF-7 for estrogen receptors (ER) and progesterone receptors (PgR), and SK-BR-3 for Topoisomerase II alpha (Topo2a), were used for IHC of invasive breast cancer tissues resected or biopsied at our institute between March 2010 and October 2011. The Levey-Jennings (LJ) test (JMP 9, SAS Institute Inc., Cary, NC) was used to examine 99.7% confidence intervals of IHC [1]. MCF-7 KU812 and SK-BR-3 were co-stained with breast tissue samples as IHC control. IHC using these systems and antibodies against ER (SP1, Roche, 1:1), PgR (1E2, Roche, 1:1), and Topo2a (Ki-S1, Dako, 1:800) was performed as described previously [2, 3]. All slides with control cell lines and breast cancer tissues were scanned and filed digitally for WSI and each digital images was automatically analyzed to count the positive rates of cell lines and cancer cells for antibodies used as described previously [1, 2]. The use of breast cancer samples for this study was approved by the Institutional Ethic Board at the Kure Medical Center/Chugoku Cancer Center (NHO-KureH141129).

RESULTS

IHC for ER was performed using 156 samples (biopsy 78, resected samples 78). For PgR, there were 223 samples (biopsy 110, resected samples 112). For Ki67 and Topo2a, they were 238 samples (biopsy 114, resected samples 123). Upper and lower limits of confidence intervals of positive rates were determined by LJ test analyzing cell lines on 100 slides. These results were 101.22% and 78.07% for ER, 59.69% and 10.12% for PgR, and 108.30% and 29.93% for Topo2a

(Table **1**). Data obtained on all slides analyzed showed that eight control cell-line samples exceeded the upper and lower limits of confidence intervals. IHC was performed again on these samples, and each control cell lines showed positive rates within the intervals in the second IHC. The positive rate scores of these breast cancer samples showed no change compared to both positive rates (Table **2**).

Table 1. Upper and lower limits of immunohistochemistry results for cell lines by the Levey-Jennings test (n=100).

	ER	PgR	Topo2a
UCL	101.2%	59.7%	108.3%
LCL	78.1%	10.1%	29.9%

UCL, upper control limit; LCL, lower control limit.

Table 2. The Levey-Jennings test and assessment of breast cancer tissue for immnohistochemistry.

Antibody	Control limit (%)	Positive rate of control cell lines(%)			Positive rate score of samples(%)	
		first	difference	second	first	second
ER	78.07～101.22	76.76	- 1.31	96.64	3+	3+
PgR	10.12～59.69	62.92	+ 3.23	60.05	1+	1+
PgR		61.13	+ 1.47	54.23	0	0
PgR		61.00	+ 1.31	55.10	2+	2+
PgR		63.58	+ 3.89	58.92	1+	1+
PgR		60.71	+ 1.02	49.00	2+	2+
PgR		66.83	+ 7.14	45.40	1+	1+
Topo2a	29.93～108.3	24.62	- 5.31	45.33	2.42%	1.94%

CONCLUSION

The current system utilizing AS, WSI, computer software and cell lines can reduce the deviation at each IHC step. Data obtained with this system is objective

SUBJECT INDEX

D

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X